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US ARMY INSTITUTE OF SURGICAL RESEARCH

PROCEEDINGS

OF THE

40th ANNIVERSARY SYMPOSIUM

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ORGANIZER: BASIL A. PRUITT, Jr., MD, FACS

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In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 86-23.

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INTRODUCTORY COMMENTS

FOUR DECADES OF PROGRESS IN BURN CARE AND RESEARCH

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THE US ARMY Institute of Surgical Research, then known as the Surgical Research Unit, was moved from Halloran Army Hospital on Staten Island to Brooke General Hospital at Fort Sam Houston in 1947. The unit, under the leadership of its first Commander, Colonel Edwin J. Pulaski, initially maintained its primary focus on the study of surgical infections as an extension of its World War II activities, defining the role of the then newly discovered antibiotic, penicillin, in the treatment of war wounds. Driven by the realization that thermonuclear weapons generated large numbers of burn injuries, Medical Corps planners then directed that the Unit establish a burn center where burn patient care could be provided and the pathophysiology of burn injury could be studied. The Unit received its first burn patient in 1949 and has since then cared for 7,549 burn patients and firmly secured its national and international leadership position in burn care, teaching, and research.

At the end of each decade since 1947, staff and alumni have met to review accomplishments and progress and, more importantly, identify remaining or new problems to which clinical and research efforts can be applied to effect continuing improvement in the care of burn patients. On behalf of the present staff members of the Institute, it is a pleasure to welcome our alumni to the Institute's 40th Anniversary Symposium. It is a special honor to have with us many of the members of The Surgeon General's Advisory Committee on the Metabolism of Trauma who, during the formative years of the Institute, provided wise counsel and perceptive scientific review and, during the past four decades, have been loyal proponents of the Institute and its members at the national and international level. The past 40 years can be viewed as a continuum of steady progress in our understanding of burn injury and resultant improvement in burn care. The following review consists of a decennial update of the Institute's activities with emphasis placed on advances and improvements that have occurred since the time of the 30th Anniversary Symposium held here in San Antonio in 1977.

Clinical Activities. It is essential that a burn center admit sufficient burn patients to permit valid clinical studies. In the past decade, the Institute has maintained patient density by admitting 2,220 burn patients. The mean extent of burn during the past decade has been 31.61% of the total body surface area, confirming that the Institute has continued to function as the military's tertiary referral

center for burn patients. The distribution of burn size and comorbid factors, such as inhalation injury, within that population has provided an appropriate depth and breadth of clinical experience to the medical students, house officers, and "fellows" assigned to the Institute. The distribution of burn size in the population is depicted in Figure 1. Burn size-related mortality, also indicated in that figure, has been nil in patients with burns of 10% or less of the total body surface area, universal in patients with burns of more than 90% of the total body surface area, and increased in dose-response fashion between those two extremes.

In addition to in-hospital care, the Institute continues to carry out, in collaboration with the United States Air Force, aeromedical transfer of burn patients. During the past decade, 51% of burn admissions (1,133 patients) were aeromedically transferred to the Institute. Eight hundred and seventy-seven flights within the continental United States and 21 flights to and from locations outside the continental United States were necessary to transfer 1,061 and 72 burn patients, respectively. Six additional toxic epidermal necrolysis patients were transferred by aeromedical means. During those burn flights, there was one in-flight death of a patient deemed moribund prior to transfer from an overseas hospital.

Facility Renovations. Extensive modernization of the clinical facilities of the Institute during the past decade has increased the efficiency of the Clinical Division. In 1983, completely renovated critical care and intermediate care sections were opened. The intensive care section now has 9 individual patient rooms with full monitoring capability, individualized temperature control, and infrared heat shields for patient warming. Each patient is visible from a centrally sited nursing station. The 4-room intermediate care section, consisting of three 2-bed rooms and one 1-bed room, has individual room temperature control as well as monitoring and warming capability for each bed. In 1987, the convalescent ward was renovated and modernized with maintenance of the multi-bed room format to retain the necessary mobilization flexibility. Each bed has the full monitoring capability necessary for use as an intensive care bed.

The metabolic study room was also renovated to permit continuation of the Institute's metabolic research program. Several sections of the laboratory have also been renovated. The microbiology and physiology sections have been modernized and the animal care facilities have been upgraded in anticipation of AALAC approval. A lightproof room has been constructed for endocrine studies and the laboratory operating facilities have been modernized to accommodate planned large animal studies.

Investigative Activities. The Institute's research activities remain focused on the response to and complications

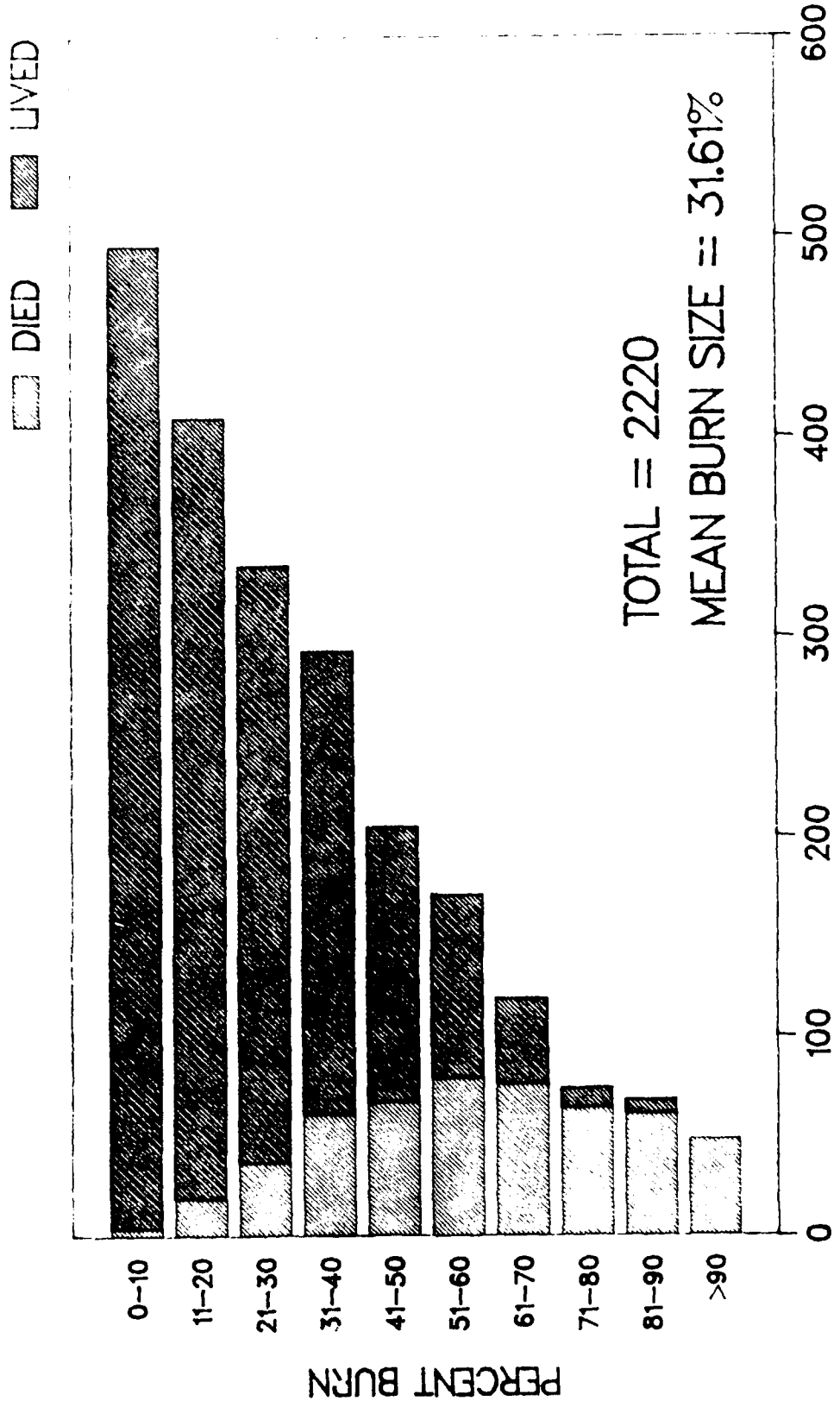

FIGURE 1. Burn Admissions (1977-86)

of severe injury as exemplified by extensive burns. Staff members are presently conducting research in the fields of hemodynamics and shock, the pulmonary response to injury, wound care, infection and sepsis, host resistance, the metabolic response to injury, and postinjury nutrition. Recent accomplishments of those investigative projects include development of an ovine model simulating burn-induced hypovolemia by use of plasmapheresis, development of a dose-responsive ovine model of inhalation injury in which multiple inert gas elimination assay of $\dot{V}_A/Q$ has identified airway injury as the predominant early change, a clinical study demonstrating the usefulness of a newly available skin substitute, epidemiologic confirmation of the virtual disappearance of Pseudomonas infection and emergence of candidal infections in severely burned patients, chemilumigenic probe identification of the prognostic significance of decreased neutrophil membrane oxidase activity, identification of the differential effects of burn injury and infection on lymphocyte subpopulations, identification of the effects of acute burn injury on central nervous system neuronal populations, and definition of the effects of burn injury on zinc metabolism.

In addition to the specific research projects, Dr. Mason and others have developed several computerized burn information data bases (Table I). Of particular note is the outcome analysis program, the use of which has confirmed the importance of comorbid factors such as inhalation injury and pneumonia. The outcome analysis program has also been used, on request, to evaluate the clinical results of other burn centers. Other programs have been developed to automate microbiologic

TABLE I. USAISR Development of Computerized Burn Information Data Base

1. Processing and reporting of laboratory results
2. Microbial surveillance system
   a. Microorganism identification
   b. Antibiotic sensitivities
   c. Epidemiologic reviews
3. Nutritional monitoring
4. Burn patient outcome analysis

surveillance, nutritional monitoring, and the reporting and correlative display of clinical chemistry results.

Educational Activities. The educational activities of the Institute have expanded markedly during the past decade. In addition to the traditional medical student and resident rotations, in accord with memoranda of agreement with 84 medical schools and other educational programs, a de facto burn fellowship program has been established for university-based PGY-3 or PGY-4 surgery residents. During this past decade, 112 medical students have taken 1- or 2-month rotations at the Institute, as have 202 military residents and 59 residents from civilian programs. Fifty-seven residents and fully trained surgeons have taken electives of more than 3 months' duration or have completed "burn fellowships" of 6 to 24 months' duration at the Institute. These latter individuals have included 14 military surgeons, 9 civilian surgeons, and 34 surgeons from foreign countries, i.e., 7 from Norway, 4 each from Japan and the Dominican Republic, 3 each from Israel, Belgium, and Jordan, 2 from the Philippines, and 1 each from Canada, Yugoslavia, Hong Kong, Panama, Venezuela, Pakistan, Malaysia, and Egypt.

Publications. In addition to patient outcome, the publication of the results of our scientific endeavors is another quantifiable end point of the Institute's activities. During the past decade, members of the Institute have published 235 articles in refereed medical journals, 105 textbook chapters, and 5 books. Among the books is a new major textbook of surgery that has received enthusiastic reviews.

Institute Accomplishments. In preparation for this 40th Anniversary Symposium gathering, our current staff has reviewed the clinical and laboratory research that has been conducted during the past four decades and have identified, on the basis of publications and identifiable changes in patient care, advances in both clinical care and the understanding of burn injury that have emanated from this Institute. The accomplishments listed in Tables II through V represent the fruits of the labors of both the alumni and current staff members of this burn center whose individual research projects contributed to the advances listed.

During its 40 years of experience, the Institute has been continuously favored by a professional staff whose outstanding characteristics, in addition to loyalty to the Institute, have been enthusiasm, dedication to patient care, and investigative excellence. The Institute has followed the progress of our alumni with great interest and is proud to recognize at this symposium their academic achievements and continued presence in burn care throughout the nation. Thirteen of our alumni have reached the academic pinnacle of chairmanship of medical school departments, 7 in surgery, 2 in anesthesiology, and 1 each in urology, pediatric surgery, internal medicine, emergency

TABLE II. USAISR Clinical Care and Research Accomplishments

1. Exposure treatment reintroduced
2. Development of aeromedical transfer teams and procedures
3. Formulation of "Brooke Formula"
4. Prophylactic hemodialysis for acute renal failure
5. Early studies of burn wound excision
6. Identification and definition of burn wound sepsis
7. Validated use of temporary biologic dressings
8. Definition of pathogenic factors in wound infections and sepsis
9. Development of topical antimicrobial therapy
10. Description of acclimatization mechanisms
11. Validation of crystalloid resuscitation
12. Development of surveillance and biopsy monitoring techniques
13. Definition of necessary structure of skin substitute
14. Description of postburn pulmonary pathophysiology
15. Validated techniques to diagnose inhalation injury
16. Description of natural history of stress ulcer
17. Development of effective stress ulcer prophylaxis
18. Description of pathophysiology of electric injury
19. Identification of "stress" lesions of lower gastrointestinal tract
20. Environmental control of infection in burn patients
21. Identification of humoral indicators of infection
22. Evaluation of "new" topical agents
23. Clinical evaluations of new biologic dressings and skin substitutes
24. Evaluation of inhalation injury therapy
25. Documentation of antibacterial and wound healing effects of direct current

TABLE III. USAISR Clinical Care and Research Accomplishments: Definition of Metabolic Effects of Burn Injury

1. Validation of intrinsic nature of hypermetabolism
2. Definition of local wound changes
3. Description of neurohormonal response
4. Identification of altered nutrient utilization
5. Description of mitochondrial response
6. Identification of injury-infection interactions
7. Development of nutritional regimens
8. Evaluation of pharmacologic treatments

TABLE IV. USAISR Clinical Care and Research Accomplishments: Definition of Immunologic Changes in Burn Patients

1. Definition of effects of burn size and depth
2. Postburn immunoglobulin changes
3. Lymphocyte changes
   a. Altered function
   b. Subpopulation reproportioning
4. Neutrophil changes
   a. Altered function
   b. Changes in distribution
5. Evaluation of immunomodulators

medicine, physiology, and biology. Sixty other alumni have attained the rank of professor and 25 the rank of associate professor. In addition, 33 of our alumni are present or past directors of burn centers or burn units and 17 others have staff appointments at burn centers or units.

TABLE V. USAISR Clinical Care and Research Accomplishments: Development and Use of Animal Models

1. Primate model of acute renal failure
2. Murine models of burn wound infection and sepsis
3. Canine model of burn shock
4. Porcine model of hypertrophic scar
5. Murine model of stress ulcers
6. Murine model of electric injury
7. Large and small animal models of postburn injury metabolism
8. Ovine model of inhalation injury

It is a special honor to recognize this four-decade report of achievement by the Institute as a whole and the individual accomplishments of the Institute's staff and alumni and a personal pleasure to welcome all of you to this symposium marking the 40th anniversary of this Institute with which I have been associated for 24 of those years. Our past work and that which will be reported during this symposium has unquestionably redounded to the benefit of injured patients and, even more importantly, has formed the basis for those studies that, during the next decade, will further advance care of the injured soldier and increase our understanding of the pathophysiology of injury.

CHAPTER I - SYSTEMIC CHANGES

EDEMA FORMATION FOLLOWING BURN INJURY

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IN ORDER TO investigate the effects of thermal injury on microvascular hemodynamics and permeability, hindpaw arterial (P_A), venous (P_V), and capillary (P_C) pressures, blood (Q_B) and lymph (Q_L) flow, and lymph (C_l) and plasma (C_p) total protein concentrations were measured before and for 3 h after a 10-sec 100°C scald burn in 11 dogs.

MATERIALS AND METHODS

Prior to injury, in 8 experiments (Group I - permeability analysis), PV was elevated by outflow restriction until the minimal C_l/C_p was obtained. In 3 experiments (Group II - hemodynamic analysis), outflow was not restricted. Lymph and plasma protein fractions ranging in size from 37 to 120 A were measured using gradient gel electrophoresis and capillary equivalent pore sizes were calculated. In the early postburn period, P_C increased from 24 ± 2 (mean ± SE) to 47 ± 5 mmHg ($P < 0.05$) and precapillary resistance (R_A) decreased from 6.6 ± 0.2 to 2.5 ± 0.2 mmHg/ml/min/100 g ($P < 0.05$), while postcapillary resistance (R_V) remained unchanged. Pre- to postcapillary resistance (R_A/R_V) fell by 74%. The reflection coefficient for total proteins (calculated as $= 1 - C_l/C_p$) decreased from 0.87 ± 0.01 to 0.45 ± 0.02 ($P < 0.01$). Permeability of the postburn capillary endothelium was described by using two populations of equivalent pores. Preburn pore radii were 50 and 300 Å, with 13% of the capillary filtrate passing through the large pores. Pore radii increased after injury to 70 and 400 Å, with 49% of the filtrate passing through the large pores. The postburn total tissue filtration coefficient (K_f) increased to 2.4 times the control. Over the first 3 h postburn, 53% of the increase in capillary filtration was attributable to increased capillary pressure and 47% to increased permeability. It is concluded that the early rapid edema formation following thermal injury is the result of marked increases in both capillary filtration pressure and filtration through large nonsieving pores.

In a second group of experiments, burns were performed in mechanically ventilated mongrel dogs under sodium pentobarbital anesthesia (30 mg/kg IV). In Group I (n = 4), 3 L of 100°C water were poured over the shaved flank, back, and side, resulting in an approximate 40% total body surface area burn.

Animals were resuscitated with lactated Ringer's solution at a rate of 2 ml/kg/% burn over the first 6 h postburn. Animals in Group II (n = 5) received a 100°C scald burn to the left paw. In both groups, cannulas were placed in the jugular vein, carotid artery, femoral artery, and a branch of the lateral saphenous vein catheters just above the left ankle. The arterial and saphenous vein catheters were connected to calibrated pressure transducers (Statham Model P23) and pressures were recorded (Grass Model 7D polygraph). A cannula was placed in a prenodal lymphatic just above the ankle and Q_L was measured using calibrated micropipets. Lymph and venous blood were collected in heparinized tubes and C_l and C_p concentrations were measured using a calibrated protein refractometer. P_C was measured by the venous occlusion technique. R_A/R_V was calculated as $P_A - P_C - P_V$, where P_A is femoral artery pressure, P_C is capillary pressure, and P_V is saphenous vein pressure.

RESULTS

We observed no changes in the reflection coefficient in nonburned skin. While there was a 10-mmHg fall in the effective oncotic gradient, we observed no change in lymph flow, indicating that there was no net change in microvascular filtration. The calculated filtration coefficient decreased, suggesting that the fall in the oncotic gradient was offset by a decrease in the microvascular surface area available for fluid exchange. Burned tissue was characterized by a marked decrease in the reflection coefficient and an increase in the filtration coefficient, indicating a marked increase in microvascular permeability. Filtration was further favored by a 16.9-mmHg fall in the effective oncotic gradient. Finally, capillary pressures were significantly increased for the first 3 h postburn and accounted for greater than 50% of the total increase in filtration.

DISCUSSION

The value of the experimental model presented here is that it allows us to separate the various factors affecting microvascular filtration. Specifically, one can separate pressure from permeability effects and thus more completely evaluate the role of mediators in postburn edema formation.

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FLUID RESUSCITATION OF ADULT BURN PATIENTS

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SINCE THE CONCEPTS of adequate volume resuscitation with balanced salt solution and temporal administration of plasma expansion (colloid) were generally accepted and widely practiced, the early mortality from major thermal injury has dramatically improved. The two "guidelines" to resuscitation embodying these principles are the Parkland formula and the modified Brooke formula. The former suggests a calculated volume of 4 ml/kg/% burn while the latter suggests an initial volume of 3 ml/kg/%. Both emphasize adjusting the projected rate in individual patients. While both rely on clinical signs of normal urinary output, cerebral function, and chemical balance, the Parkland formula further emphasizes a rapid, complete restoration of cardiac output and cardiovascular integrity as essential to optimal therapy.

Analysis of the results of resuscitation using the Parkland formula is shown in Table I, representing all acute admissions in a metropolitan area over a 5-yr period. Fifty-nine patients were deemed "nonresuscitatable" by reason of incineration, prearrival cardiac arrest, and cerebral death. Of the 55 "resuscitation failures," two-thirds (or 1.04% of the entire population) died within 48 h. Since deaths within the first week could potentially be related to resuscitation, these are included as resuscitation failures, although most occurred from early sepsis, inhalation injury, and disease-specific causes.

TABLE I. Parkland Memorial Hospital Burn Center Mortality Data (1982-87)

	Number of Patients (% of Total Acute Admissions)
Acute admissions	2352
Total early deaths	114 (4.8)
Nonresuscitated	59 (2.5)
Deaths - Rx (< 8 days)	55 (2.3)
< 48 hr	32 (1.7)
> 48 hr	22 (0.9)

Table II shows that all failures of resuscitation in adult patients occurred in young adults with moderate and massive burns and in the elderly with moderate burns. Approximately 20% of the young adults with burns >85% of the total body surface area were resuscitation failures, while almost 35% of the elderly with a mean burn size of 52% were resuscitation failures. This data emphasizes the increasing systemic effect of cutaneous injury and the limited capacity of the aging individual to withstand massive insults. Successful resuscitations in both categories have been reported by Monafo and Caldwell using hypertonic saline solutions, but no data is available to compare survival percentages.

TABLE II. Parkland Memorial Hospital Burn Center - Early Deaths (1982-87)

Age Group	Number of Deaths	Average Burn Size (%)
Children (< 5 yr)	10	58
Adults (17-46 yr)	21	85
Aged (> 65 yr)	24	52

Current investigative thrusts involve the addition of pharmacologic agents directed toward effecting or altering the increased capillary permeability or oxygen radical scavenging, but none have been sufficiently developed to permit clinical trials.

Investigations over the past two decades have contributed to the improved clinical care of patients during this important initial interval and have demonstrated that the systemic response manifested in immunosuppression, hypermetabolism, and abnormal wound healing actually begins within the first few hours after injury.

The response of "burn shock" to fluid resuscitation is often decreased in very extensive burns. The cardiac output is slower to return to normal as the burn size increases. By 10-h postburn, patients with total body surface area burns <40% reached a cardiac index of 5-1/2 $L/m^2/min$, while patients with burns >40% often did not obtain this level until 20 h postresuscitation. Oxygen consumption doubles in larger burns within the first 10 h, while lesser burns require several more hours to obtain their ultimate maximum. A definite myocardial effect of burn injury is present and related to the size of injury. In larger burns, contractility is diminished and both systolic and diastolic compliance falls, resulting in a higher ejection fraction and elevated end-diastolic filling pressures.

These lesions become clinically important in the massive burn and in the large burn in elderly patients. Study in aged animals has shown that coronary vasodilatation partially restores both parameters to a survivable level.

The reduced efficiency of the cell wall sodium pump mechanisms was initially assumed to be secondary to the adenosine triphosphate-derived (fast) components. This default was first demonstrated in response to hemorrhagic shock and was correctable by volume resuscitation. Diminished activity was also found to occur within 1 to 1-1/2 h after burn shock in man. Membrane function in burns, however, is not restored and intracellular sodium concentration remains between 2 and 4 times greater than that of normal muscle cells. The results are explainable only as an increase in the permeability of cells to sodium, which is calculated as between 3 and 5 times greater than normal. The importance of this observation clinically is that extracellular fluid can remain depleted while clinical parameters, such as weight of the patient, imply adequate isotonic volume in this space. This finding negates the use of weight as an index of volume resuscitation.

Other evidence of cell wall abnormalities were shown in the etiology of burn anemia. Beginning at the time of injury, the release of stored fatty acids results in high circulating levels that affect the elasticity of red cell membranes and in a severe reduction of red cell survival time. Evidence for oxidative stress exists in the rapid depletion of glutathione reductase, which adds to cell wall structural abnormalities and becomes an additional factor in red cell survival.

Systemic peroxidation of lipids is evidenced by elevated levels of malondialdehyde and other hydroperoxides derived from essential fatty acid breakdown. Additional studies have shown an inability to raise the level of arachidonic acid in most burn patients until after the second to third week postburn. The demonatration of decreased total cholesterol to approximately 50%, with most of the reduction occurring in the esterified cholesterol fraction, lead to the demonstration of abnormalities in most lipoprotein subclasses. Some of the more important ones are the very low concentrations of HDL and quantitative alterations in LDL. This results in reduced delivery of cholesterol to the cells and the lack of availability to remove cholesterol from the cells. The severe reduction in CII apoprotein compromises the host's handling of chylomicrons which is evident in the high circulating levels of triglycerides found in most major burns.

Demonstration of these and other systemic abnormalities attenuates our classic visualization of burn shock as a fluid-dependent phenomenon, existing for 40-60 h after injury, and emphasizes the systemic organ and cellular effects that contribute not only to the burn shock phase, but to the host's

immunosuppression and hypermetabolism that are hallmarks of the major burn.

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FLUID RESUSCITATION OF BURNED CHILDREN

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THERMAL CUTANEOUS injury produces changes in virtually every organ system of the body, resulting in a variety of systemic responses. The most immediat consideration in the early treatment of a burned child relates to changes in fluid and electrolyte status. The larger the injury, the greater the magnitude of fluid shift. This review describes current methods of fluid replacement applicable to children with severe thermal injury. It should be noted that virtually all of the methods used to estimate fluid requirements can be made to work, but some are easier to use than others. This is of interest, particularly since each of the formulas differs from the others with respect to the amount and composition of the fluid administered, the rate of administration, or the sequence in which fluids are administered.

The ideal method of fluid resuscitation in burned children has not been developed since all the elements of the pathophysiologic response to burn injury are not yet known. Regardless of the method used to estimate fluid requirements, the rate of fluid administration should be sufficient to produce acceptable hourly urine flow and adequate cardiac output based on clinical guidelines. Some patients with very large burns will require central venous pressure and pulmonary capillary wedge pressure monitoring. However, the majority of burned children may have cardiac output estimated quite accurately by assessing the adequacy of peripheral circulation, maintenance of normal sensorium, normal blood pH, and normal body temperature. Burn formulas are not very helpful for children with burns <20% of the total body surface area and most of these patients can adequately be treated with a combination of oral and intravenous fluids.

Regardless of the fluid approach used, the sine qua non of successful resuscitation as pointed out by Baxter is somewhere in the vicinity of 0.52 mEq of sodium/kg/% burn. This tends to correlate with acceptable support of cardiac output and urine flow. However, during the first 24 h postburn, it would appear to be impossible to completely restore plasma volume to normal by any currently available regimen. At any rate, while the composition of various fluid regimens may vary in terms of sodium, the total amount administered does not appear to vary a great deal. Additionally, it would appear to be more beneficial to constantly administer uniform amounts of sodium and water rather than to vary solutions with different sodium

concentrations in sequence. Stable resuscitation regimens appear to produce stable patient responses.

In our studies and in those performed at the US Army Institute of Surgical Research, a linear relationship has been noted between the amount of surface area burned and the amount of fluid required for satisfactory resuscitation. While patients with burns in the range of 25-35% of the total body surface area had fluid needs in our studies in the range of 3 ml/kg/% burn as described by the new Brooke formula, those with larger injuries were more effectively treated by the Parkland regimen or with even more fluids. This would appear to be related to the greater magnitude of metabolic rate and insensible water loss associated with a larger surface area to body weight ratio in children as compared with older subjects. It was of further interest to note that patients with inhalation injuries or very deep and extensive full-thickness burns required additional amounts of fluid replacement in a nonlinear fashion. While none of our patients developed evidence of even mild pulmonary edema, diuretics were often used to accelerate the rate of water excretion.

Colloid in the form of 5% albumin is generally beneficial when administered after capillary leak has abated during the second 24 h postburn. There is no general agreement as to whether earlier administration is possibly beneficial or not, but we have not encountered any particular disadvantage by withholding it for the first 24 h postburn. The new Brooke and Parkland formulas would appear to be the most practical and most economical approach to fluid resuscitation in the burned child, reserving colloid for the second postburn day. We initiate therapy at 3 ml/kg/% burn and rapidly increase fluids as determined by the patient's response. Regardless of the method used to estimate fluid requirements, preservation of normal organ function and restoration of normal physiology must be the goal of resuscitation.

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BOVINE HEMOGLOBIN SOLUTIONS

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THE INCREASING shortage of blood and fear of blood-transmissible diseases have given a sense of urgency to the development of a relatively safe and efficient blood substitute. Hemoglobin in solution has long been considered for this purpose, since it is capable of performing two essential functions, i.e., maintaining intravascular volume and transporting oxygen in quantities similar to that by intact red blood cells.

Removal of hemoglobin from erythrocytes, however, creates a number of problems, some of which are still unresolved. Two major problems identified in the 1970s were the high oxygen affinity of hemoglobin in solution resulting from loss of 2,3-diphosphoglycerate and short intravascular persistence of the solutions due to dissociation of the hemoglobin tetramer into its subunits. The former was solved by modification of the hemoglobin molecule with pyridoxal-phosphate to increase P-50, and the latter by polymerizing hemoglobin subunits with glutaraldehyde (1-2). The stability of these "poly-hemoglobins" has not been proven, however, and the use of glutaraldehyde may have introduced a new element of toxicity (3).

A third problem was the increasing shortage of outdated blood needed to prepare hemoglobin solutions; blood banks had simply become "too efficient" in the storage and utilization of blood products. As a result, we proposed the use of bovine hemoglobin as an alternative (1983), based on the gross similarities of molecular structures, better oxygen delivery characteristics without the need for 2,3-diphosphoglycerate (oxygen affinity of unmodified bovine hemoglobin solution similar to that in intact human red blood cells), the fact that hemoglobin itself is not antigenic, and the obvious large scale availability. Experience with the use of polymerized bovine hemoglobin solutions during the past 4 years has shown them to be capable of remaining in circulation for a prolonged period of time while retaining the ability to transport and unload oxygen in a normal manner.

A fourth and more complex problem is the toxicity of these solutions, where studies have produced conflicting results. Rabiner et al popularized the idea that hemoglobin free of stroma residues is essentially free of toxicity (4), while others maintain that hemoglobin is intrinsically toxic, independent of the degree of purity (5-7). This variability of findings can be attributed to at least two factors: the use of

many different animal models and experimental protocols and hemoglobin solutions with variable levels of purity. Although hemoglobin almost completely free of insoluble stromal elements can be produced by a variety of techniques, the solutions continue to produce toxicity.

MATERIALS AND METHODS

Recently we investigated four potential factors of toxicity, red blood cell phospholipids, "environmental" endotoxins, large molecular aggregates resulting from the polymerization of hemoglobin with glutaraldehyde, and hemoglobin itself. Using a bovine hemoglobin solution prepared by ultrafiltration (8) in a group of monkeys, we found that contamination with stromal phospholipids, endotoxin, or hemoglobin polymers in excess of 500 kDa MW activate complement via the alternate pathway (9). Toxicity of phospholipids is specifically due to the presence of phosphatidyl-enthanolamine (PE) and -serine (PS) which are located on the cytoplasmic side of the cell membrane and appear to have a strong affinity for the hemoglobin molecule. Endotoxins also have a high affinity for hemoglobin, and it is difficult to prepare biological solutions that are not contaminated with endotoxin in most research laboratories. Hemoglobin solutions polymerized with glutaraldehyde are not stable at 4°C and form progressively larger polymers with the passage of time. Finally, hemoglobin itself has a tendency to auto-oxidize and generate free oxygen radicals which are expected to exhibit toxicity (10). Clinically, the monkeys uniformly exhibited mild hypotension and occasional PVCs during infusion of the solution, but thereafter, appeared normal.

To further test toxicity in a more sensitive animal model, a bovine hemoglobin solution polymerized with glutaraldehyde was prepared (8). Four modified solutions were prepared from this basic solution using a combination of techniques, treatment with chloroform to remove residual stroma phospholipids, filtration through a Detoxi-Gel column to remove endotoxins, and storage of the solution at 4°C for 1 month to allow the development of large hemoglobin polymers. Bovine polymerized hemoglobin solution #4 (BPSH-4) represented the "ideal solution" since it was purified to the maximally attainable degree in our laboratory and stored at -20°C until use. The solution designated BPHS-3 contained no stromal phospholipids or endotoxin, but the majority of hemoglobin polymers had MW exceeding 500 kDa. BPHS-2 contained 0.50 endoxtoxin U/ml (assessed by the quantitative chromogenic limulus test), but did not contain stromal phopholipids or high MW hemoglobin polymers. BPHS-1 contained PE and PS, but was free of endotoxin and high MW hemoglobin polymers. The solutions were tested in 4 groups of 6 rabbits each by isovolemic replacement of one-third of the calculated blood volume; a fifth group received homologous rabbit plasma instead of hemoglobin solution and served as controls.

RESULTS

Response to the infused solutions in the first 3 groups (BPHS 1-3) were similar. Immediate reactions during infusion included hypotension, cardiac arrhythmias, bronchospasm with severe hypoxia, and hemoglobinuria. Laboratory data showed an immediate thrombocytopenia and leukopenia out of proportion to the degree of hemodilution, a marked reduction in creatinine clearance, elevation of SGPT, activation of the alternate complement pathway, and findings consistent with severe disseminated intravascular coagulation. One-third of the animals in each group died during or shortly after the infusion of the hemoglobin solution, and the remainder were sacrificed at 24 h postinfusion. Gross pathologic findings included hemoglobin extravasation into pleural and peritoneal cavities, marked congestion and edema of the lungs, liver, and kidneys, and multiple hemorrhagic infarcts in the spleen. Histologic examination showed severe ischemic-inflammatory changes in these organs, with widespread microthrombosis and marked mononuclear cell infiltrates. In contast, BPHS-4 and the control group did not show a rise in any component of complement and did not produce any change in blood elements, coagulation factors, or fibrin-split products. Histologically, only mild ischemic changes were found in the myocardium, liver, and kidneys in the BPHS-4 group.

DISCUSSION

The results of these studies suggest that the toxicity of hemoglobin solutions, as currently prepared, is due principally to "impurities" such as stromal phospholipids (particularly PE and PS), contaminating endotoxins, and large hemoglobin polymers. These impurities activate the alternate complement pathway and cause a series of systemic reactions which have been collectively called "acute inflammation of the blood" (11-12). Hemoglobin free of these impurities still causes mild toxicity, which may be due to the release of free oxygen radicals during auto-oxidation to methemoglobin (10,13-14).

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GASTROINTESTINAL RESPONSE TO INJURY

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THE GASTROINTESTINAL tract can be a focal point for major injurious sequelae of trauma. Specifically, the mucosal surface of the proximal stomach is the area of the gut most often affected. The multiple superficial erosions seen lying above the muscularis mucosa are most often referred to as stress ulcers. Clinical risk factors correlated with this injury have included multiple trauma, burns, hypotensive shock, renal failure, jaundice, sepsis and peritonitis, respiratory failure, and major operative procedures (1). If preventative steps are not initiated in such patients, anywhere from 10% (in uncomplicated trauma) to >50% (in severe blood loss or sepsis) may be affected by the major hemorrhage that can accompany the deepening of these lesions into the rich submucosal vascular network. While massive blood loss accounts for only a small fraction of the mortality seen in patients grossly bleeding from stress ulcers (10%), fully half of such patients eventually succumb to their underlying pathology.

Despite the volume of investigative research that has taken place in the past decade, no single etiology of stress ulcer formation has been agreed upon. Rather, a list of several pathophysiological determinants has been generated, including the presence of luminal gastric acid, histamine stimulation, the secretory status of the gastric mucosa (acid, mucus, and bicarbonate), mucosal energy status, permeability, and acid-base balance, mucosal blood flow, and prostaglandin activity within the gastric mucosa (2). Changes in any one or combination of these are now thought to be crucial to the initiation of stress ulceration.

The prostaglandins, because of their significant concentrations within gastric mucosa and paracrine mode of action, have garnered much attention. Most, especially PGIs and Es, possess several actions having clear cytoprotective value. Among others, these include the ability to increase bicarbonate and mucus production, mucosal blood flow, and high energy compounds within gastric lining cells while increasing gastric acid secretion (3). Specific studies in this laboratory have demonstrated the gross cytoprotective action of exogenously administered prostaglandin compounds (4). Additional investigations have delineated the necessity of an effect on mucosal blood for prostaglandin cytoprotection to succeed (5). Unfortunately, almost all of the studies dealing with prostaglandin cytoprotection have utilized exogenous, pharmacologic doses to achieve their results. The lack of a simple, reproducible method for the measurement of physiologic

levels of tissue prostaglandin generation has been a major restriction. More recently, the technological evolution of HPLC has allowed such determinations to be carried out routinely (6). This, in turn, has permitted the performance of studies that assess the physiologic role of endogenous prostaglandins in the cytoprotective process. The following represents the first in a series of such studies performed in our laboratory and used either vagotomy (a known stimulant of prostaglandin generation) or the cyclooxygenase inhibitor indomethacin (a known suppressant of prostaglandin synthesis) to alter endogenous mucosal prostaglandins.

MATERIALS AND METHODS

One hundred twenty-eight Sprague-Dawley rats weighing approximately 250 g were used. All animals were anesthetized and underwent celiotomy via midline incision. Half the animals had a total, bilateral truncal vagotomy performed while the other half served as sham-operated controls. Postoperatively, all animals were allowed a 7-day recovery period, during the last 48 h of which solid food was withheld (water given ad libitum). One h prior to study, each group of 64 rats (sham-operated group and vagotomy group) were further divided equally into subgroups that received either saline or indomethacin (30 mg/kg IP). Thus, the following 4 study groups of 32 rats each were formed: Group 1, sham-operated/saline; Group 2, vagotomy only/saline; Group 3, sham-operated/indomethacin; and Group 4, vagotomy/indomethacin. Each group was then subjected to cold-restraint stress (wire meshed wrapped at 4°C) for 0, 1, 2, or 4 h (n = 8 rats for each time period). At the conclusion of the stress period, each animal was sacrificed and the stomach was removed, washed, and photographed for later assessment of injury by blinded observers using a predetermined scoring system based on total area of erosion in the glandular mucosa. Concomitantly, a biopsy of corpus mucosa was surgically excised, weighed, and incubated in 2 ml buffered saline for 4 h at 37°C. The mucosal tissue was then removed, sonically disrupted, and underwent prostaglandin extraction. The PGI_2 content (measured as the stable analogue PG 6-keto-$F_{1\alpha}$) of each pair of tissue extract/incubation media samples was then determined by specific HPLC analysis.

RESULTS

Results of injury assessment are presented in Table I. Injury scores increased along with the duration of cold-restraint stress in all groups except Group 4. In Group 2, injury was significantly less than that seen in Group 1 during the period of most severe stress (4 h). However, there was no significant difference in injury scores between those two groups at lesser periods of stress (0, 1, and 2 h). Group 3 evidenced significantly greater injury scores at all stress

TABLE I. Comparison of Glandular Mucosal Injury Scores in Animals Subjected to Cold-Restraint Stress (Mean ± SE)

Group[a]	Stress Duration (h)			
	0	1	2	4
1	0.0±0.0	0.8±0.7	5.6±0.7[b]	27.3±3.1[b]
2	0.0±0.0	0.4±0.3	5.5±0.6[b]	10.9±0.9[b,c]
3	8.4±2.9[d]	34.0±2.5[b,c,d]	62.9±4.7[b,c,d]	79.8±3.5[b,c,d]
4	0.0±0.0	4.8±0.4[b,c]	3.8±0.6[b]	4.5±0.2[b,c]

[a] n = 8 per stress period for each group.
[b] $P < 0.05$ when compared to time = 0 within group.
[c] $P < 0.05$ vs. Group 1, same time.
[d] $P < 0.05$ vs. Groups 2 and 4, same time.

durations than those seen in Group 1. Finally, Group 4 had injury scores during the most severe stress period (4 h) that were significantly less than those seen in the equally stressed Group 1. However, at stress durations less than 4 h, the Group 4 had injury scores similar to those of Group 1.

Results of mucosal prostaglandin-generating capacity are presented in Table II. Severe cold-restraint stress (4 h) was associated with significant decreases in PGI_2 generation in both Groups 1 and 2 to levels that were not significantly dissimilar. Group 2 evidenced significantly greater PGI_2 generation than did Group 1 at 0, 1, and 2 h of stress. Both indomethacin groups (with or without vagotomy) had significantly less PGI_2 generation at 0, 1, or 2 h stress than did either Groups 1 or 2. Furthermore, the PGI_2 generation remained unaffected by any stress duration in both indomethacin groups. Finally, the PGI_2 generation levels in all 4 study groups were similar after 4 h stress.

DISCUSSION

These results demonstrate that cytoprotection can be achieved during severe cold-restraint stress (in vagotomy only and vagotomy/indomethacin groups) despite a prostaglandin-generating capacity diminished to the level seen in the sham-operated control group animals. Physiologic increases in prostaglandin generation (vagotomy only at 1 and 2 h stress) over that seen in the sham-operated control group do not necessarily inhibit initiation of gross mucosal injury. The relationship of prostaglandin generation to gastric acid

TABLE II. Comparison of PG 6-Keto-$F_{1\alpha}$ Corpus Muscosal-Generating Capacity (pg/mg Mucosa) (Mean ± SE)

Group[a]	Duration of Cold-Restraint Stress (h)			
	0	1	2	4
1	577 ± 10	568 ± 14	550 ± 7	255 ± 10[b]
2	691 ± 13[c,d]	683 ± 18[c,d]	652 ± 7[c,d]	306 ± 10[b]
3	310 ± 28[c]	317 ± 29[c]	318 ± 24[c]	354 ± 12
4	374 ± 24[c]	323 ± 20[c]	286 ± 21[c]	291 ± 15

[a] n = 8 per stress period for each group.
[b] $P < 0.05$ wnen compared to time = 0 within group.
[c] $P < 0.05$ vs. Group 1, same time.
[d] $P < 0.05$ vs. Groups 2 and 4, same time.

secretion, visceral neural input, and nonacid gastric secretions remains to be fully defined. However, these results at least call into question a primary physiologic role of endogenous gastric mucosal prostaglandins in the cytoprotective process.

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VASOMOTOR NEPHROPATHY OF INJURY

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Ischemic Renal Injury. The initiating event which leads to cell destruction is an increase in cell membrane permeability. Ischemia activates phospholipase, which destroys the lipid bilayer of the cell membrane. This results in an influx of calcium into the cell. Once calcium begins entering the cell, it rapidly accumulates over a very short period of time. Normally, calcium is located within one of three pools within the cell. Ten to 20% is bound to the plasma membrane, approximately 60-70% is sequestered in intracellular organelles, and 10-20% is free within the cytosol (1). It is the free calcium which is biologically active. Calcium has numerous properties, but those which are germane to cell injury include its ability to activate membrane phospholipase, its ability to alter active transport processes in the membrane, and its effect on cytoskeletal structural conformations (2-3). Ischemia may also result in a redistribution of intracellular calcium pools with an increase in the cytosol of the free (biologically active) form. Increased mitochondrial calcium results in decreased respiratory function and a reduced rate of generation of adenosine triphosphate (ATP). The impaired energy production reduces the ability of the active transport process to extrude the influx of calcium from the cell. Calmodulin, which is a calcium regulatory protein present in most cells, is important in controlling phospholipase activity (4). It may also be altered during ischemia.

Ischemia also results in the generation of free radicals. Free radicals and calcium work in concert to destroy lipids and thereby destroy the membranes. Free radicals may activate or inhibit enzymes, and they also alter cell function by reacting with intracellulr molecules (5). Free radicals are generated under conditions of normal respiration by the oxidation of NADPH to NADP. Two oxygen-free radicals plus a hydrogen ion yields hydrogen peroxide, and hydrogen peroxide plus an oxygen-free radical in the presence of iron yields a hydroxyl-free radical (6). The free radicals produced under circumstances of normal respiration may damage the cell; however, their concentration is kept at a minimum by the naturally occurring free radical scavenger glutathione and by the enzyme glutathione peroxidase. In the presence of a limited oxygen supply, however, the mitochondria are required to increase their workload, and the production of free radicals markedly increases. Free radicals induce lipid peroxidation and, in the presence of calcium, cause mitochondrial membrane dysfunction (5). Another mechanism of free radical generation occurs when high energy phosphates (ATP) are degraded to

adenosine monophosphate and subsequently to hypoxanthine. In the presence of ischemia, regeneration of ATP is limited and hypoxanthine accumulates. During reperfusion or when oxygen is supplied, hypoxanthine is converted to xanthine by xanthine oxidase, which results in the generation of an oxygen-free radical. Thus, restoration of blood flow may be injurious, in that it increases the production of free radicals just as the limitation of blood flow and ischemia results in an increased production of free radicals, the latter through the mitochondrial respiratory mechanism and the former through the degradation of high energy phosphate compounds.

The cellular events which follow ischemic injury may be summarized as follows. Ischemia activates phospholipase which degrades the lipid bilayer of the cell membrane and increases its permeability. Continued action of the phospholipase results in membrane disruption. Also, toxic lipid by-products from membrane degradation potentiate further membrane destruction. Increased membrane permeability allows extracellular calcium to diffuse into the cell. The intracellular calcium enhances the activity of phospholipase, thus further destroying the membrane and increasing its permeability. As intracellular calcium increases and diffuses into the mitochondria, there is a disruption of mitochondrial respiration, lack of energy production, and increased free radical production, all of which combine to further destroy the membrane. Calcium, in conjunction with free radicals, injures mitochondria and reduces cellular respiration. Not only may the membrane be destroyed by these mechanisms during the ischemic event, but its destruction may also be enhanced when the ischemia is corrected and reperfusion established. This occurs as ATP is reduced to adenosine monophosphate, which then degrades to hypoxanthine during the ischemic episode. Hypoxanthine accumulates. When the cell is reoxygenated, xanthine oxidase is stimulated to convert hypoxanthine to xanthine. This produces an oxygen-free radical which attacks the membrane and destroys its structure.

Hormonal Alterations During Renal Ischemia. The renin-angiotensin system is activated during renal ischemia and plays a significant role in altering both blood flow and cellular response. Renin is produced in the juxtaglomerular cells of the juxtaglomerular apparatus, either as a response to decreased arteriolar pressure or a decreased distal tubule sodium concentration sensed by the macula densa. Stimulation of the sympathetic nervous system, various prostaglandins and calcium also stimulate renin production. Renin is produced in its inactive form, which is activated by a serum protease. Activated renin acts on renin substrate, an alpha globulin produced by the liver to cleave the decapeptide angiotensin I. Converting enzyme, ubiquitously present, cleaves two amino acids from angiotensin I to form the octapeptide angiotensin II. Angiotensin II is a very potent vasoconstrictor and its production inhibits the secretion of renin. Angiotensin

stimulates aldosterone production, which enhances distal tubule sodium reabsorption as well as distal tubular potassium secretion. It may directly affect the production of the prostaglandin PGE_2, a vasodilator. This system regulates glomerular filtration rate through its effect noted above on the arteriole, and it may result in peripheral and central vasoconstriction (7).

The eicosanoids or the products of arachiodonic acid metabolism are the other hormones which play a prominent role in renal ischemia. There are three main groups of products of arachidonic acid metabolism. If arachidonic acid is acted upon by the enzyme cyclooxygenase, endoperoxides are produced. Most tissues have the capability of producing the endoperoxides; however, the enzymes which further facilitate the production of specifc prostaglandins are unique to particular tissues. Within the kidney, the endoperoxides may be converted to prostacyclin (PGI_2), PGE_2, PGF_2, or thromboxane A_2. The renal medulla is the most active site of prostaglandin synthesis. The prostaglandins regulate renal blood flow and glomerular filtration rate, and they tend to maintain these in the presence of vasoconstriction. They inhibit the action of antidiuretic hormone, decrease thick ascending limb transport of sodium and chloride, interfere with urea reabsorption in the tubular epithelium, and also increase medullary blood flow. Prostaglandins most involved in these aspects of concentration and dilution are PGE_2 and PGF_2. They may also play a role in erythropoietin production (8).

The second group of products of arachidonic acid metabolism are the leukotrienes. These substances are formed when the enzyme lipoxygenase acts upon arachidonic acid to produce 5-HPETE. From this substance, the leukotrienes are formed. The leukotrienes, although not indigenous to the kidney, are prominent within polymorphonuclear leukocytes. They are vasoconstrictors and increase membrane permeability. They are particularly active as chemotactic factors and, when introduced into the kidney, reduce the glomerular filtration rate. The kidney may, however, be involved in the metabolism of the leukotrienes (9-10). The third group of products of the eicosanoids are the hydroxyl fatty acids, which are produced as a result of the enzyme epoxygenase. These substances inhibit sodium transport (8).

These two hormonal systems, renin-angiotensin and the eicosanoids, work in concert, one affecting the other. Thus, when the juxtaglomerular cells produce renin and subsequently generate angiotnesin II, further output of renin is inhibited by the direct action of angiotensin II upon the juxtaglomerular cells. Angiontensin II results in vasoconstriction which, in addition to its direct action, activates phospholipase. Phospholipase activation results in an increased production of arachidonic acid and thereby, in the presence of

cyclooxygenase, an increased amount of endoperoxides. This results in the kidney in an increased production of prostacylcin and PGE_2, which directly stimulate the juxtaglomerular cells to increase renin production. Thus, these hormones work in concert, one stimulating the other and one performing the opposite action of the other. Under normal physiologic conditions, fine regulation of intrarenal blood flow and glomerular filtration rate is maintained by the appropriate production of these two hormonal products. During ischemia, however, this balance is disrupted and their vasoconstrictive properties can be unopposed, thus making the ischemia worse and prolonging its effects.

Prevention and Treatment of Ischemic Acute Renal Failure. The modalities used to treat and/or prevent acute renal failure following ischemia may be divided into five groups, those which alter environment, drugs which are used to manipulate blood flow, urine flow, and/or transport processes, manipulations of the renal hormones, manipulations of the free radicals, and alterations in energy provision and manipulations of their metabolites. Environmental alterations involve manipulation of the core temperature of the kidney. Drugs utilized to alter blood flow, urine flow, and sodium transport include mannitol, dopamine, and furosemide. Alterations of the renal hormonal system may be accomplished with drugs such as captopril or by altering the sodium content of the diet. Free radical production may be reduced by enzyme inhibitors such as allopurinol, or the detrimental effect of free radicals can be diminished by the administration of scavengers. Free radical scavengers include those which occur naturally, such as glutathione, as well as those which may be produced exogenously, such as dimethylthiourea, catalase, and superoxide dismutase. Alterations in energy provision include drugs which provide energy as ATP-magnesium chloride and inosine or drugs such as theophylline which directly interfere with the vasoconstrictive metabolites of high energy compounds.

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SUMMARY OF CHAPTER I - SYSTEMIC CHANGES

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RECOGNIZING THAT formation of edema in the burned area is the initial, salient event of burn injury, this session appropriately began with Dr. Curreri's discussion of its relevant mechanisms. A burn model in dogs in which the transcapillary Starling forces can be directly measured, in addition to sizing of proteins in the lymphatic effluents, demonstrated that burn edema forms because transcapillary pressures rise (afferent arteriolar dilatation with fixed efferent resistances), oncotic gradients are reduced, and capillary permeability increases, without significant changes in nonburned areas.

The next two presentations logically addressed fluid resuscitation in burned adults and in burned children. Dr. Baxter's retrospective summary of more than 5,000 patients indicated a more rapid restoration of cardiovascular function using 4 rather than 3 ml/kg/% burn of electrolyte solutions and the relative lethality of older age and larger burns.

Multiple studies in these patients demonstrated the early onset and progression of such additional systemic sequelae as immunosuppression, hypermetabolism (with time-to-peak varying directly with the size of the burn), abnormal wound healing, reduced myocardial contractility and compliance, increased muscle cell permeability to sodium with reduced sodium outflux pumping rates, reduced erythrocyte survival, and abnormalities in cholesterol and triglyceride transport and metabolism. Dr. O'Neill agreed that burn resuscitation formulae must be guided by individual responses of urinary flow and cardiac output, emphasizing that the enhanced metabolic rates and larger body surface-to-volume ratios in children spoke for use of larger resuscitation volumes. The latter would necessarily be modulated by the reduced concentrating and diluting functions of these patients' less mature kidneys.

Addressing two problems, colloids for resuscitation and burn anemia, some of the properties of bovine hemoglobin solutions as blood substitutes were reviewed by Dr. Canizaro. Toxicities due to residual erythrocyte phospholipids, endotoxin, and larger hemoglobin polymers that activate complement and induce circulatory changes appeared related to the method of preparation, while purification of hemoglobin solutions seemed to confer greater stability and less toxicity.

The continuing problems of "stress ulcer" in the proximal one-third of the stomach were next addressed by Dr. Levine in terms of a possible cytoprotective effect of prostaglandins. In a model of cold-stress in rats, however, truncal vagotomy was protective despite indomethacin-induced reductions of prostaglandin-generating capacity. This militated against prostaglandins as mediators of protection.

Finally, Dr. McDougall considered possible mechanisms of ischemic renal cell injury in burned patients that might also imply useful treatments. The injurious sequences include disruption of cell membranes, and of mitochondrial respiration with toxic oxygen-free radical production, activation of both the renin-angiotensin system, and of the counterbalancing and interactive effects of eicosanoids, endoperoxides, leukotrienes, and hydroxyl fatty acids. These mechanisms suggested that potentially useful treatment modalities to prevent renal failure following burn injury might include modificiations of core temperature and use of such drugs as mannitol, dopamine, furosemide, captopril, allopurinol, and several free radical scavengers.

In this as in other parts of this Symposium, and since the beginning of the Institute 40 years ago, important trends are evident. Empiricism ("what works") is increasingly replaced by measurements and mechanistic descriptions and those descriptors are increasingly sought at progressively lower levels of biological organization, i.e., tissues, cells, and molecular biology. Perhaps the alumni of the Institute do not need to be reminded of the risky tradeoffs, that the requirements for desirable measurements (good) can make artifacts (bad) and that "explanations" sought and found among molecules must somehow retraverse increasingly complex interactions at progressively higher levels of biological organization in order to reemerge for the benefit of the whole organisms that presented the clinical problem in the first place.

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THE ELUCIDATION of the systemic consequences of burn trauma has focused not just on the pathophysiology of the wound, but also on the response of major organ systems. This effort has been paralleled by a similar quest to develop therapeutic techniques that will not only provide for multisystem support, but will also override or negate those responses that have an overall detrimental effect. Despite advances in many areas, controversy still exists with respect to many aspects of the burn wound and of the various therapeutic techniques utilized in the clinical setting.

In this part, the contributing authors discuss many of the more clinically relevant controversial issues. Beginning with the wound itself, Dr. Curreri highlighted the pathophysiology of burn edema and describes an experimental model that provides a means for evaluating the effect of various mediators on this basic, but still not completely understood, component of burn injury. This discussion was followed by discussions which focused on the topics of fluid resuscitation in the adult and pediatric age groups. Dr. Baxter reaffirmed the importance of aggressive fluid resuscitation as a prime determinant of early survival and went on to define many of the associated system abnormalities as a fluid-dependent phenomenon. The effect on varied organ systems was discussed in conjunction with the theoretical concept of a pharmacological agent that will have the propensity for altering capillary permeability and oxygen radical scavenging. This discussion was complimented by Dr. O'Neill's discussion of the resucitation of burned children, introduced by his caveat that "the ideal method of fluid resuscitation in burned children has not been developed." He went on to highlight the importance of standardizing sodium content of solutions utilized for volume replacement in this age group. Additional discussion on resuscitation was presented by Dr. Canizaro, who discussed the advantages and disadvantages of utilizing a bovine hemoglobin solution as a blood substitute. Problems of toxicity were defined and will serve as a basis for increased investigational work on this important and timely topic. Finally, attention was directed to two specific target organs. Dr. Levine continued his work on the gastrointestinal response, while Dr. McDougal further highlighted the vasomotor nephropathy associated with burn injury. Both authors clearly and concisely discussed the changes occurring in the gastrointestinal and renal systems and then went on to discuss effective therapeutic techniques. The authors laid the groundwork for further investigational efforts centered around the endocrine manipulation of their respective organ systems.

Collectively, the discussions highlighted many of the important facets of the systemic nature of burn trauma. Discussions included not only an elucidation of the pathophysiology of burn edema, but also the techniques of volume replacement and protection of target organ systems. This broad scope of information ran the gamut from the cellular response to the generalized organ response which can be expected in victims of burn trauma.

CHAPTER II - PULMONARY CHANGES

DIAGNOSIS OF INHALATION INJURY

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INHALATION INJURY is a surface insult to the tracheobronchial tree secondary to breathing in noxious gases which produce a chemical burn of the mucosal lining. The terms used to describe this syndrome include pulmonary burn, lung burn, and smoke inhalation. The extent and depth of this pulmonary injury depends upon the duration of exposure and the type of toxic gas. Three clinical presentations are commonly seen in inhalation injury and correlate to the anatomical levels of injury. These include upper airway, major airway, and parenchymal inhalation injuries (Fig 1). The first presentation includes the development of stridor, hoarseness and progressive upper airway occlusion, primarily during the first 24 h, and involves damage to the larynx down to the level of the true vocal cords. Damage to the major airway includes the trachea and primary bronchi and produces clinical manifestations such as wheezing, bronchospasm, bronchorrhea, and a productive cough. This major airway inhalation injury is frequently complicated by superimposed infection in the form of tracheal bronchitis or pneumonia. This complication occurs primarily in the second to the fifth day postinjury. The third type of inhalation injury involves damage to the alveolar space and produces an irreversible hypoxia manifested with ARDS. It is evident immediately following the thermal injury.

CLINICAL FINDINGS

Prior to the development of objective diagnostic testing, a series of clinical criteria were used to diagnose the presence of inhalation injury. This criteria included physical findings of facial burns, burned vibrissae, changes in chest auscultation, bronchorrhea, and sooty sputum and historical facts of a closed space accident, the presence of thick smoke, and a history of unconsciousness. These findings, as shown in Table I, had a variable sense of correlation to inhalation injury. However, two or more of these findings were considered highly suggestive of inhalation injury. Because the development of physical findings is delayed 24-48 h after injury, therapeutic interventions were limited, and inhalation injury in the preobjective diagnostic era was associated with a high mortality rate.

Initial laboratory data is also not always helpful. The admission chest X-ray is very insensitive in determining pulmonary injury. Pulmonary edema is evident in 50% of patients with inhalation injury, again occurring 24-36 h postinjury. It is not uncommon to have admission arterial

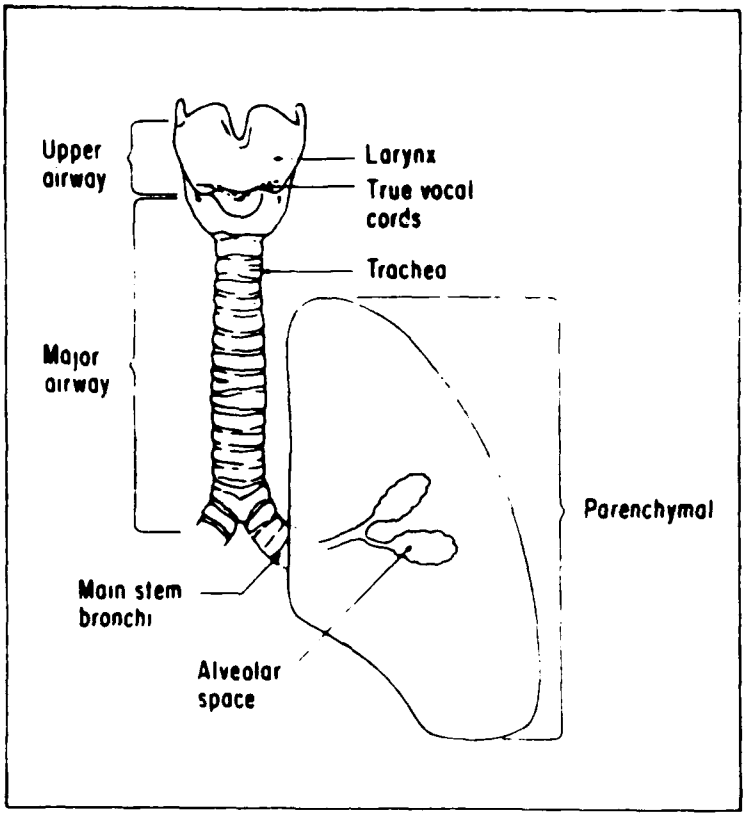

Figure 1. Anatomical levels of injury.

blood gases within the normal range in over 80% of patients with pulmonary damage.

DIAGNOSTIC TESTS

Xenon 133 Lung Scan. Objective diagnostic testing for inhalation injury began approximately 15 yr ago. In 1972, xenon 133 lung scanning was employed at the US Army Institute of Surgical Research (San Antonio, TX) for the diagnosis of inhalation injury. Intravenous administration of xenon gas with serial scintophotograms provided objective evaluation of the components of respiration, including ventilation abnormalities associated with airway damage. Since this isotope is excreted almost entirely into the alveoli during the first passage through the lung, this lung scan provides a semiquantitative measurement of ventilatory clearance from each aspect of the lung. Delay in clearing of the isotope or asymmetrical clearance of the gas is associated with airway injury. Clinical experience with this technique found it to be safe and accurate and performed without the necessity of the patient's cooperation. Primary limitations of this technique have been the relatively short half-life of the isotope, making

TABLE I. Physical Findings and Historical Facts Used in Diagnosis of Inhalation Injury

Physical Findings	Frequency
Facial burns	70%
Auscultatory chest abnormality	20%
Burned vibrissae	13%
Bronchorrhea and sooty sputum	10%

Historical Facts	Frequency
Unconsciousness	80%
Closed space accident	75%
Thick smoke	25%

its availability limited. There was a potential for both positive and negative examinations because of preexisting pulmonary diseases such as bronchitis and bronchiectasis secondary to smoking or asthma. However, extensive experience with this technique has shown a very low incidence of false interpretation (Table II).

Flexible Bronchoscopy. Use of the fiberoptic bronchoscope provided another diagnostic modality for the early diagnosis of inhalation injury and was reported by the University of Wisconsin Burn Center as well as the US Army Institute of Surgical Research. This bedside technique using the fiberoptic bronchoscope and topical anesthesia has become a standard throughout many burn centers in the United States because of the widespread availability of this instrument. Criteria for inhalation injury includes laryngeal or tracheal inflammation, edema, necrosis, and ulceration of the airway as well as the presence of soot or scarring in the airway itself. The technique can be performed without complication if certain steps are used, including complete topical anesthesia of the nasal and pharyngeal mucosa with an agent such as 1% mepivacaine, adequate preoxygenation of the patient with 100% oxygen, and supplementation of oxygen through a side port during the procedure itself in those patients who have mild hypoxia. In addition, if a nasotracheal tube is placed over the bronchoscope prior to the diagnostic test, the tube can be placed into the airway under direct visualization in those patients with a high potential for upper airway obstruction. This test has continued to be highly accurate in the diagnosis of inhalation injury with very few false negatives.

TABLE II. Results of Pulmonary Mechanics in Patients with Inhalation Injury (Mean ± SD)*

	Inhalation Injury (+ Xenon 133 Scan)	Controls	P Value
VC (% predicted)	80.8 (15.6)	85.3 (19.4)	NS
FRC (% predicted)	96.0 (18.3)	89.6 (22.1)	NS
TLC (% predicted)	76.0 (13.3)	80.5 (13.5)	NS
Peak flow (% predicted)	61.9 (17.0)	99.1 (15.1)	< 0.01
Flow at 50% VC (% predicted)	41.6 (14.3)	98.7 (25.7)	< 0.01
C_{STAT} (L/cm H_2O)	0.281 (0.113)	0.324 (0.159)	NS
C_{DYN} (L/cm H_2O)	0.290 (0.160)	0.224 (0.188)	NS
R_{PULM} (cm H_2O/L/sec)	4.85 (0.31)	3.08 (1.01)	< 0.01

Pulmonary Function Testing. Pulmonary function testing, including maximum expiratory flow volume (MEFV) curves, are extremely valuable, not only in the diagnosis of inhalation injury, but in determining the physiological prognosis of the injury. It has been shown that patients with objective evidence of inhalation injury by bronchoscopy or lung scan who have a normal MEFV may have a benign course, while those with objective evidence of inhalation injury and an abnormal MEFV have been associated with a high mortality rate.

SUMMARY

The incidence of inhalation injury had been reported as 2.9-11% of thermal injury patients prior to the use of objective testing. However, there was an additional group of patients, up to 20% of the burn population, who developed pneumonia or other airway abnormalities at 48-72 h postinjury. Since the use of objective testing, the incidence of inhalation injuries has remained steady, reported to be 32-38% over the last 15 yr. This includes those patients with major airway injuries who, prior to objective testing, were not initially diagnosed but who developed delayed pneumonia.

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PULMONARY VASCULAR EFFECTS OF INHALATION INJURY

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THE MORBIDITY and mortality in burned patients are significantly increased when accompanied by an inhalation injury (1). We have performed studies in an animal model which simulate the salient clinical features of inhalation injury (2-9). These studies were conducted to investigate the pathogenesis of inhalation injury in an effort to modulate the host response to this trauma.

MATERIALS AND METHODS

Range ewes (n = 78), with an average weight of 40 ± 1.2 kg, were prepared by placing a catheter in the left atrium and cauterizing the borders of the diaphragm and posterior aspect of the left thoracic cavity to sever systemic lymphatics which might enter the caudal mediastinal lymph node. One week later, the efferent lymph vessel from the caudal mediastinal lymph node was cannulated by a modification of the technique described by Staub et al (10). In all sheep, a flow-directed thermodilution Swan-Ganz catheter was positioned in the pulmonary artery via the external jugular vein and a catheter advanced through the femoral artery into the thoracic aorta. These catheters were tunneled under the skin and exteriorized with all incisions closed in a multiple-layer manner. Tracheostomies were performed 5 cm below the thyroid cartilage and a cuffed tracheostomy tube inserted. All surgical procedures were performed under halothane anesthesia and the sheep allowed 1 wk to recover.

After recovery, baseline data were collected for 2 h and the sheep again anesthetized. Smoke was produced from smoldering cotton toweling in the combustion chamber of a bee smoker and delivered at 15 ml/kg per breath through the tracheostomy tube. The duration of smoke exposure required to cause a fatality and the duration to produce a pathophysiologic change was used to construct a smoke inhalation exposure-lymph flow relationship. Three groups of 6 animals each were insufflated with multiple sequences of 8 breaths of smoke, followed by 2 min of 2% halothane and 98% oxygen. In the first group, there were 4 replications of this smoking sequence, with each sheep receiving a total of 32 breaths of smoke (light exposure). A second group received 4 replications of a 12-breath smoking sequence in the same manner (medium exposure), and the third group received an exposure of 4

replications of a 16-breath smoking sequence (heavy exposure). The light exposure group showed little pathophysiologic change, while the medium exposure group showed a significant pathophysiologic change, resulting in a 50% mortality with no ventilator support. In the heavy smoke exposure group, there was a 100% mortality, even with maximal ventilatory support. The temperature of the smoke delivered was monitored at the level of the endotracheal connector and controlled to not exceed 39°C.

In the following experiments, a nonlethal but maximal pathophysiologic exposure (medium exposure) was used. Six animals received gabexate mesilate (a proteolytic enzyme inhibitor) in a loading dose of 1 mg/kg at 30 min postinjury (PI) and 1 mg/kg/h for the next 48 h PI. Six animals received 10,000 U aerosolized heparin through the endotracheal tube every 4 h for the first 48 h PI and 6 animals received 3 ml 90% dimethyl sulfoxide (DMSO) and 10,000 U aerosolized heparin via endotracheal tube every 4 h for the first 48 h PI. Six animals received a loading dose of ibuprofen (12.5 mg/kg) 30 min PI followed by 6 mg/kg/h as a continuous infusion for the next 48 h PI. An additional group of 12 animals were depleted of white blood cells, using 0.6 mg/kg nitrogen mustard at 5 and 3 days prior to smoke inhalation. The white blood cell count was reduced from 7000 $\pm$ 2136 to 764 $\pm$ 66, neutrophils from 3162 $\pm$ 1486 to 315 $\pm$ 105, and lymphocytes from 3200 $\pm$ 1486 to 300 $\pm$ 105 cells/μl. Half of these animals were given a "medium smoke exposure" and half were ventilated with room air.

Arterial carboxyhemoglobin levels of 25 $\pm$ 5% were obtained with the medium smoke exposure. Arterial and pulmonary artery pressures were monitored during control and at various times following inhalation injury. Differential cell counts were by standard histologic staining techniques and total protein measured on a protometer. Albumin, IgG, and IgM were determined by a rocket immunoelectrophoretic technique. The consumptive depletion of antiproteinases was determined as an index of enzyme release, with the ability of plasma and lymph to inhibit protease in vitro as an index of antiprotease activity. Eicosanoid levels were determined by the content of thromboxane B_2 and the 6-keto-$PG\text{-}F_{1\alpha}$ levels in plasma and lymph, analyzed by specific RIA antibodies produced in rabbits.

RESULTS

We were able to demonstrate a smoke exposure response relationship between the duration of exposure and lung fluid formation (5) (Fig 1). A light exposure created only a minor pathophysiologic response with the medium exposure resulting in a large pathopohysiologic response with an increase in lung lymph flow ($\dot{Q}_L$) and a transvascular flux of protein. This group would experience a 50% mortality without ventilator

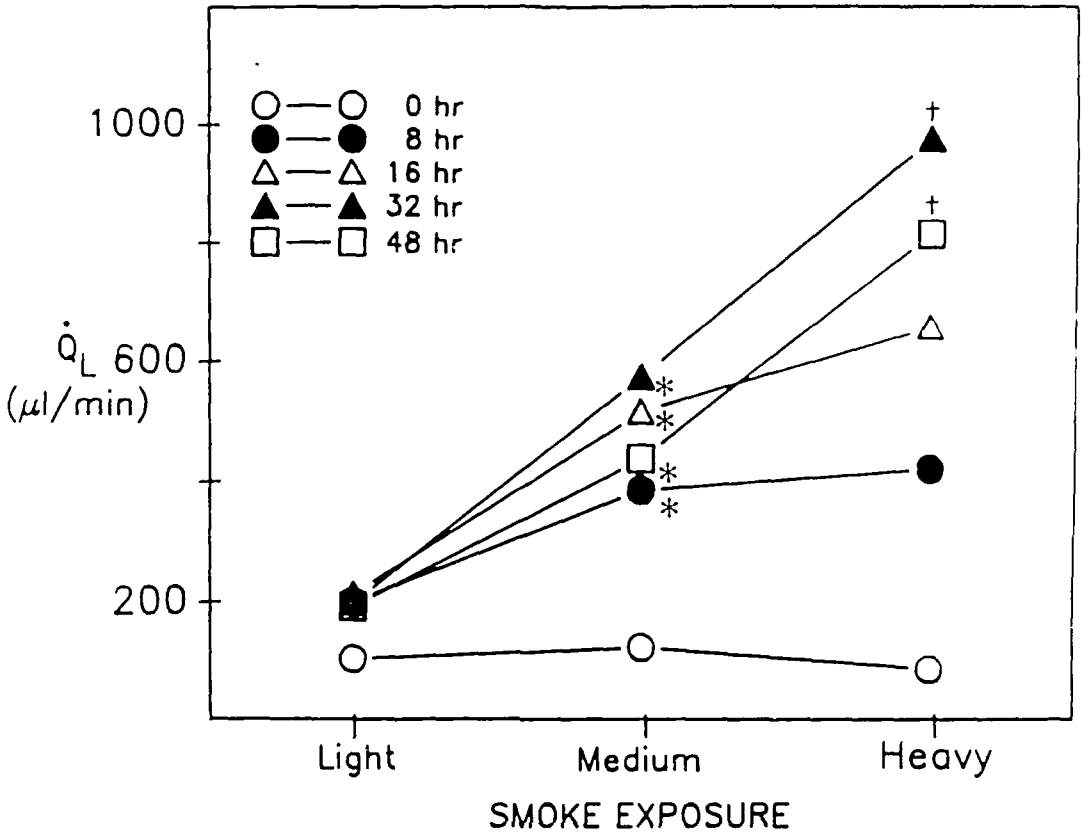

FIGURE 1. Isochronal lines depicting the relationship of smoke exposure to caudal mediastinal lymph flow ($\dot{Q}_L$). Student's t-test was used to determine differences between groups of data. $P < 0.01$ was considered signficant. *Significantly higher when compared to light exposure data. †Significantly higher when compared to medium exposure data.

support (2-4). The heavy exposure caused a marked rise in permeability and was uniformly fatal between 50-100 h PI without ventilator support. This increase in microvascular permeability was further associated with a decrease in arterial oxygenation. Cardiac indices and pulmonary artery pressures were, however, maintained within normal ranges and hypovolemia did not appear to contribute to the pulmonary fluid formation seen in these animals.

Exudate from the tracheobronchial tree was formed at a rate of approximately 3 ml/h during the first 24 h PI following moderate smoke exposure. The electrophoretic pattern of this material was similar to plasma (3). Total protein content of tracheobronchial fluid (TB) from injured animals was 4.5 ± 0.6 g/dl compared to 6.2 ± 0.2 g/dl in the plasma (P) (TB/P = 0.66

± 0.08). Albumin, IgG, and IgM ratios in the tracheobronchial fluid relative to plasma were 0.80 ± 0.03, 0.59 ± 0.07, and 0.17 ± 0.02, respectively, indicating a selective permeability. For a given microvascular permeability, there must be a change in the lymph protein concentration/plasma protein concentration (C_L/C_P) with a change in $\dot{Q}_L$. In this study, no significant change in C_L/C_P was observed with an increase in $\dot{Q}_L$ following inhalation injury. Prostacyclin and thromboxane A_2, as reflected by their stable metabolites in the tracheobronchial fluid, were 0.498 ± 0.305 ng/ml and 2.5 ± 0.9 ng/ml, respectively. The levels of thromboxane B_2 were not statistically elevated in the lymph, while 6-keto-PG-$F_{1\alpha}$ was elevated in the plasma. Large numbers of white blood cells ($5.1 \pm 0.9 \times 10^3$ cells/μl with 61 ± 11% polymorphonuclear leukocytes) were found in the tracheobronchial fluid 24-48 h PI (5). Elastase and trypsin inhibitory capacity in the lymph decreased from 130 to 90 U/ml and 750 to 600 mg, respectively.

Depletion of polymorphonuclear leukocytes (nitrogen mustard) reduced the permeability edema following smoke inhalation injury (9). Similary, treatment with DMSO (free radical scavenger), DMSO and heparin (7), and gabexate mesilate (8) each attenuated the permeability edema seen after inhalation injury (Figs 2-3). Ibuprofen, as seen in Figure 4, also ameliorates the response, in part due to its vasogenic properties (6).

DISCUSSION

A smoke inhalation injury to the sheep model described increases $\dot{Q}_L$ and transvascular protein flux with no change in C_L/C_P. We conclude from this data that an inhalation injury causes a microvascular permeability change, and further, that lung lymph flow changes are dependent upon the duration of smoke exposure. The present study demonstrates that tracheobronchial exudate, formed following smoke inhalation, is a filtrate of plasma with a composition similar to pulmonary lymph, which supports the hypothesis of bronchial microvascular damage. The presence of markedly increased levels of leukocytes and their proteolytic enzymes in lymph after inhalation injury has been demonstrated. These same phagocytic cell mediators and the potent smooth muscle constrictor, thromboxane A_2, are found in the tracheobronchial fluid following inhalation injury. Inhibition of prostanoid activity, neutrophil depletion, scavenging of free radicals, and blockade of proteolytic enzymes all decrease the pulmonary response to inhalation injury. We postulate that smoke inhalation stimulates sequestration of neutrophils in the lung, releasing proteolytic enzymes and free radicals which cause microvascular injury in both the bronchial and lung parenchymal

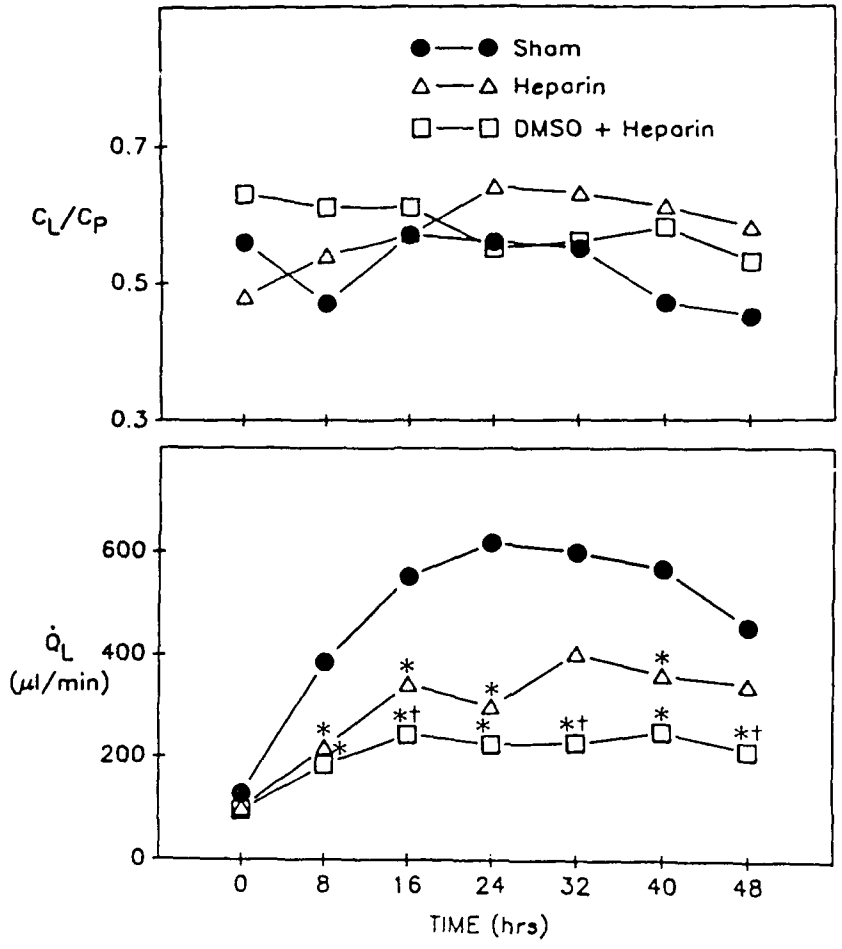

FIGURE 2. Effect of heparin and DMSO plus heparin on lung lymph flow and lymph/plasma total protein ratios following a medium smoke exposure. Ratio of caudal mediastinal lymph protein concentration to plasma protein concentration (C_L/C_P) and caudal mediastinal lymph flow rate ($\dot{Q}_L$) are plotted as a function of time. Student's t-test was used to determine differences between groups of data. $P < 0.01$ was considered signficant. *Significantly lower when compared to sham smoke data. †Significantly lower when compared to heparin treatment data.

areas. The resulting structural damage causes an increase in transvascular flux of protein and fluid. The progressive edema formation depresses pulmonary function, resulting in a decreased arterial oxygenation. These same mediators may destroy the integrity of the tracheobronchial epithelium, leading to exudate formation.

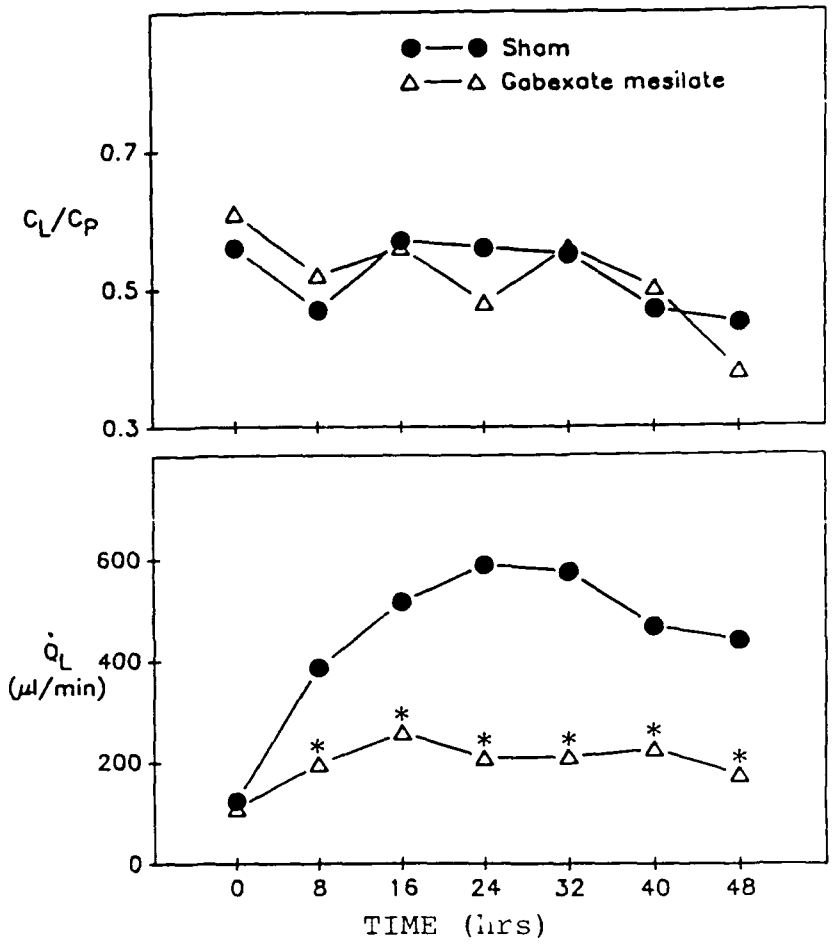

FIGURE 3. Effect of gabexate mesilate on lung lymph flow and lymph/plasma total protein ratios following a medium smoke exposure. Ratio of caudal mediastinal lymph protein concentration to plasma protein concentration (C_L/C_P) and caudal mediastinal lymph flow rate ($\dot{Q}_L$) are plotted as a function of time. Student's t-test was used to determine differences between groups of data. $P < 0.01$ was considered signficant. *Significantly lower when compared to sham smoke data.

The smoke inhalation animal model closely mimics the clinical situation where marked increases in mortality are observed when concomitant with thermal injury. The model described will be useful in modulating potential mediators of progressive dysfunction following a smoke inhalation injury.

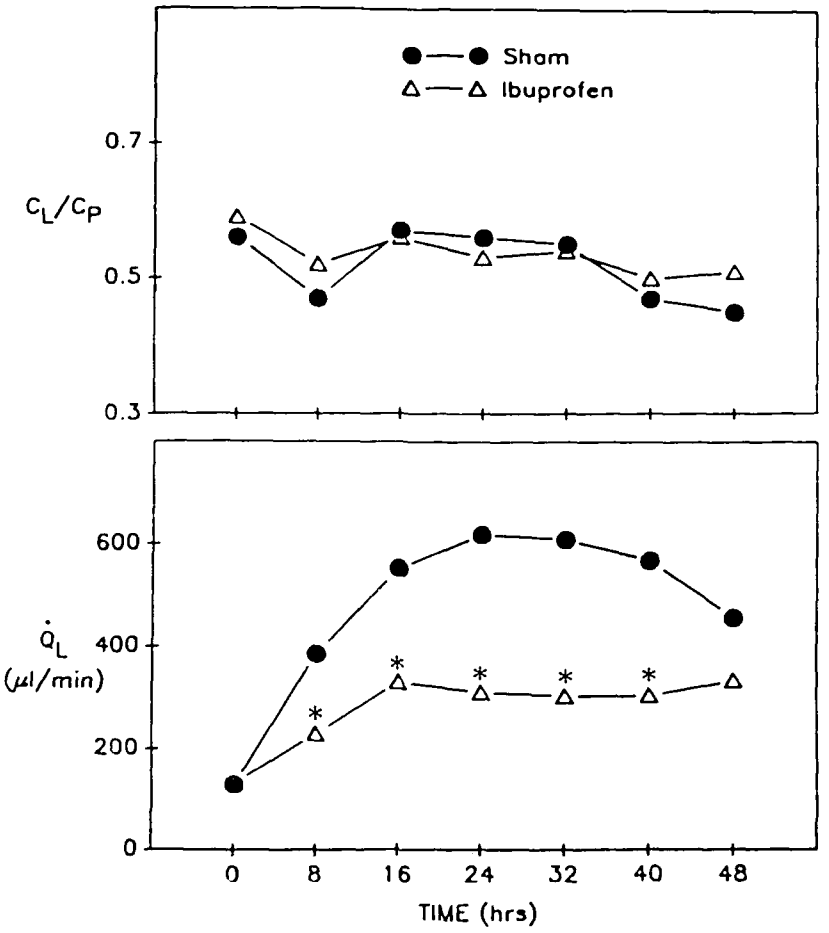

FIGURE 4. Effect of ibuprofen on lung lymph flow and lymph/plasma total protein ratios following a medium smoke exposure. Ratio of caudal mediastinal lymph protein concentration to plasma protein concentration (C_L/C_P) and caudal mediastinal lymph flow rate ($\dot{Q}_L$) are plotted as a function of time. Student's t-test was used to determine differences between groups of data. $P < 0.01$ was considered signficant. *Significantly lower when compared to sham smoke data.

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EFFECTS OF SMOKE INHALATION INJURY ON VENTILATION-PERFUSION RATIO ($\dot{V}_A/Q$) OF THE LUNG

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SMOKE INHALATION injury is one of the unsolved problems of burn care. First, early diagnosis as well as diagnosis of severity of such injury is not satisfactory in spite of recent progress in diagnositc techniques. Secondly, treatment of smoke inhalation injury is not yet established. Clinically, positive end-expiratory pressure and higher concentration of oxygen are the only regimen available for burn patients sustaining smoke inhalation injury. Thirdly, although smoke inhalation injury significantly increases mortality of burn patients, the pathophysiologic mechanism is not yet clarified. To study the pathophysiology of such injury, we have developed a reproducible and dose-responsive model and measured severity-related and time-related alterations in cardiopulmonary indices, including $\dot{V}_A/Q$.

MATERIALS AND METHODS

Forty-seven neutered male sheep were used for this study. Severity-related changes and time-related changes were separately studied.

Severity-related changes were studied in 21 sheep consisting of 5 uninjured sheep (control) and 16 sheep exposed individually to an amount of smoke which produced mild (n = 5), moderate (n = 5), or severe (n = 6) smoke inhlaation injury in a previous study (1). Cardiopulmonary indices, including $\dot{V}_A/Q$, were measured at 24 h after smoke exposure.

Time-related alterations were studied in 26 sheep, including 6 uninjured sheep (controls). Moderate smoke inhalation injury was produced in 20 sheep and measurements were made at 6, 12, 24, and 72 h after smoke exposure in groups of 5 each.

Smoke was made by burning 10 disposable underpads in a smoke generator as we described before (1). To produce smoke inhalation injury, sheep were anesthetized, intubated, and then insufflated with standard doses of smoke to produce mild, moderate, or severe injury.

Before the measurement, sheep were reanesthetized and arterial, central venous, and peripheral venous lines, a Swan-Ganz catheter, and an esophageal balloon were inserted. The animals were then positioned prone and artificially ventilated with a volume-limited ventilator. Following a 2-h

stabilization period, cardiopulmonary indices were measured. $\dot{V}_A/Q$ ratio was measured by the multiple inert gas elimination technique (2-3). We have modified the technique to apply it to sheep; krypton was used instead of the originally chosen ethane to avoid interference from endogenous ethane and a gas chromatography-mass spectrometer was used to measure krypton at a trace level (4).

Results are shown as mean ± SD. Statistical analysis was made by ANOVA (Bonferroni test) for comparison of cardiopulmonary indices. $\dot{V}_A/Q$ result was analyzed by multivariate analysis to compare fractional blood flow to the four major compartments simultaneously (5). Significance was assigned when $P < 0.05$.

RESULTS

Severity-Related Alterations. Changes in selected cardiopulmonary indices are shown in Table I. Arterial oxygen pressure (PaO_2) was significantly lower in the moderate and severe injury groups. Arterial carbon dioxide pressure ($PaCO_2$) and pulmonary resistance were significantly elevated and static compliance was significantly lower in the severe injury group. However, there was no significant difference from the control level in mean blood pressure, pulmonary artery pressure, cardiac index, total peripheral resistance index, and pulmonary vascular resistance index.

Figure 1 depicts typical $\dot{V}_A/Q$ distributions of the four groups. The distribution on the top left represents the normal, heathly animal with sharp peaks of ventilation and perfusion at $\dot{V}_A/Q$ of 1. In the mildly injured animal (bottom left), $\dot{V}_A/Q$ peaks near $\dot{V}_A/Q$ of 1 are wider and a shunt fraction (arrows) is evident. $\dot{V}_A/Q$ mismatching progressed as hypoxia became more severe with development of very low $\dot{V}_A/Q$ areas (top and bottom right).

Table II summarizes severity-related alterations in $\dot{V}_A/Q$ ratios. $\dot{V}_A/Q$ distribution was divided into four major compartments, true shunt ($\dot{V}_A/Q = 0$), low $\dot{V}_A/Q$ compartment ($0 < \dot{V}_A/Q < 0.1$), normal $\dot{V}_A/Q$ compartment ($0.1 < \dot{V}_A/Q < 10$), and high $\dot{V}_A/Q$ compartment ($10 < \dot{V}_A/Q$). Fractional blood flow to the four compartments are shown as % cardiac output. In the controls, 98.4% of the blood flow perfused normal $\dot{V}_A/Q$ compartment. Blood flow to the normal compartment decreased progressively as the injury became more severe, while flows to

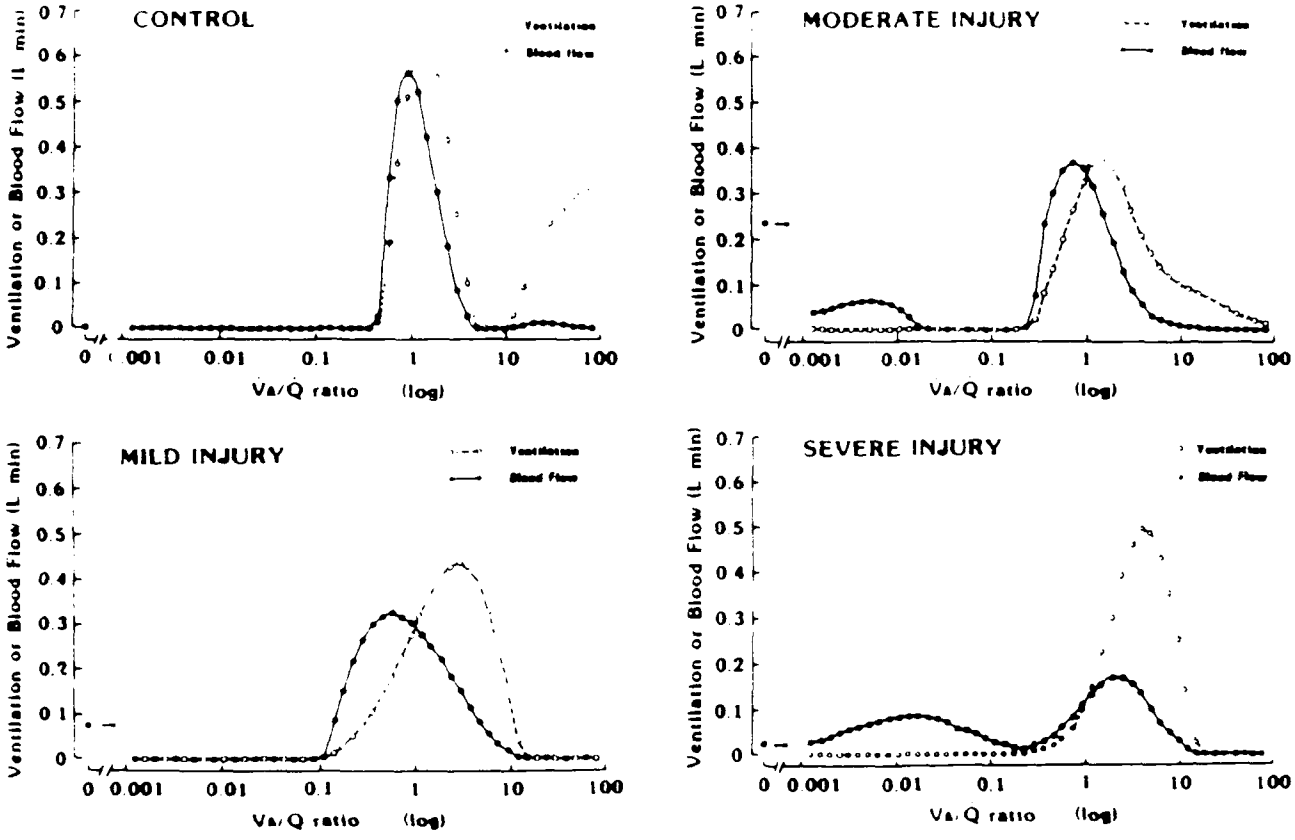

FIGURE 1. Typical $\dot{V}A/Q$ distributions of the control, mild, moderate, and severe injury at 24 h after smoke exposure are shown. Open circles represent ventilation and closed circles represent blood flow derived from parallel 50-compartment lung model. $\dot{V}A/Q$ ratio (abscissa) was plotted on a logarithmic scale.

TABLE I. Dose-Related Changes in Selected Cardiopulmonary Indices (Mean ± SD)

	PaO_2 (torr)	$PaCO_2$ (torr)	Static Compliance (ml/cm H_2O)	Pulmonary Resistance (cm H_2O sec/l)
Control (n = 5)	101 ± 6.5	31.0 ± 4.9	169 ± 24	11.6 ± 2.5
Mild Injury (n = 5)	87 ± 2.8	29.9 ± 3.2	130 ± 26	15.8 ± 5.8
Moderate Injury (n = 5)	66 ± 10.0*	40.6 ± 3.2	94 ± 35	23.8 ± 9.6
Severe Injury (n = 6)	47 ± 8.6*	47.5 ± 14.0*	52 ± 30*	52.2 ± 30.0*

*P < 0.05 by ANOVA.

TABLE II. Dose-Related Changes in the Fractional Blood Flow (Expressed as % Cardiac Output, Mean ± SD)

	Shunt	Low	Normal	High
Control	0.4 ± 0.5	0.4 ± 0.5	98.4 ± 0.4	0.8 ± 0.6
Mild Injury	0.5 ± 0.8	7.8 ± 3.6	91.3 ± 3.2	0.5 ± 0.4
Moderate Injury	5.6 ± 2.5	17.6 ± 4.8*	76.4 ± 9.3*	0.3 ± 0.2
Severe Injury	11.7 ± 9.5*	36.9 ± 4.7*	50.8 ± 9.8*	0.6 ± 0.9

*P < 0.05 from the control level by multivariate analysis.

the shunt and the low $\dot{V}A/Q$ compartment reciprocally increased. Blood flow to the normal compartment was significantly lower in the moderate and severe injury groups. Shunt flow was significantly lower in the moderate and severe injury groups. Shunt flow was significantly higher in the severe group and the low $\dot{V}_A/Q$ compartment was signficantly increased in the moderate and severe injury groups. The high $\dot{V}_A/Q$ compartment did not show any changes.

Time-Related Changes. Table III summarizes some of the cardiopulmonary indices. PaO_2 became lower with time. Static compliance and pulmonary resistance were significantly different from the control level at 72 h after smoke exposure.

Table IV shows time-related changes in fractional blood flow in the lung. Blood flow to the normal compartment decreased and flow to the low $\dot{V}_A/Q$ compartment increased with time, while shunt flow did not always increase. Blood flow to the low $\dot{V}_A/Q$ compartment significantly increased and flow to the normal compartment was lower at 72 h after injury.

DISCUSSION

Severity-related and time-related changes in cardiopulmonary indices, including $\dot{V}_A/Q$ ratios, were similar. Progressive hypoxia, lower lung compliance, and higher airway resistance were noted as time passed and as the extent of injury increased. The hypoxia and chnages in ventilatory mechanics suggest substantial change in $\dot{V}_A/Q$ distribution, which was confirmed by the multiple inert gas elimination technique. The change in $\dot{V}_A/Q$ was characterized by progressive distortion of the perfusion pattern, esperially development of very low $\dot{V}_A/Q$ compartments, which explained the etiology of hypoxia (Fig 1). Although statistical significance was not shown as a group, some of the smoke-exposed animals developed a substantial increase in shunt flow, which also contributed to the progression of hypoxia in those animals.

Robinsin et al showed increase of low $\dot{V}_A/Q$ compartment in burn patients with smoke inhalation injury at 48-72 h after injury (6). Although cardiac output in those patients are affected by burn injury and fluid resuscitation, contribution of true shunt to progressive hypoxia was limited, which was similar to our results. Thus, $\dot{V}_A/Q$ changes following smoke inhalation was characterized by development of low $\dot{V}_A/Q$ compartments. Such physiological changes are consistent with

TABLE III. Time-Related Changes in Selected Cardiopulmonary Indices (Mean ± SD)

	PaO_2 (torr)	$PaCO_2$ (torr)	Static Compliance (ml/cm H_2O)	Pulmonary Resistance (cm H_2O sec/l)
Control (n = 6)	101 ± 6.3	34.1 ± 4.1	180 ± 49	10.3 ± 2.3
6 h (n = 5)	90 ± 9.2	32.2 ± 2.8	138 ± 30	12.4 ± 1.8
12 h (n = 5)	78 ± 13.0*	33.8 ± 5.1	138 ± 55	19.1 ± 9.8
24 h (n = 5)	69 ± 9.2*	33.8 ± 3.3	110 ± 27	19.4 ± 10.5
72 h (n = 5)	64 ± 12.0*	37.3 ± 9.3	75 ± 22*	37.2 ± 14.0*

*$P < 0.05$ by ANOVA.

TABLE IV. Time-Related Changes in the Fractional Blood Flow (Expressed as % Cardiac Output, Mean ± SD)

	Shunt	Low	Normal	High
Control	0.3 ± 0.1	0	98.7 ± 0.2	1.1 ± 0.2
6 h	3.3 ± 2.2	3.2 ± 3.2	92.2 ± 2.8	1.3 ± 0.4
12 h	2.2 ± 1.6	13.6 ± 5.7	83.5 ± 6.8	0.8 ± 0.2
24 h	1.6 ± 1.2	14.9 ± 4.2	82.5 ± 4.4	0.9 ± 0.5
72 h	3.6 ± 3.0	24.6 ± 9.6*	70.4 ± 7.7*	1.4 ± 0.4

*$P < 0.05$ from the control level by multivariate analysis.

the histologic finding of extensive occlusion of the small airways.

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FLUID BALANCE IN THE LUNG FOLLOWING INJURY

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SMOKE EXPOSURE severe enough to cause inhalation injury, increases the mortality experience of burn victims significantly (1-3). The acute pathophysiologic responses to severe smoke inhalation are not well characterized. This report will review some of the pulmonary changes which acutely influence extravascular lung water following an inhalation injury in an effort to define the ways in which the smoke-damaged lung is made vulnerable to additional stress. The injury produced in this dog model is severe; no animal has survived more than 48 h following 5 min of smoke exposure, and older animals frequently die during the initial insult. All the changes noted are seen between 1 and 4 h following smoke exposure.

A standard dose of smoke, generated from the ignition of plywood sawdust and kerosene, is delivered at 37°C to the airway of mongrel dogs by a volume respirator. Smoke exposure lasts for 5 min at an average respiratory rate of 15 breaths/min. Five cm of positive end-expiratory pressure are added to the system when the chest is open; dogs are ventilated with room air prior to and following smoke exposure. Gross changes in the lung are often seen after as few as 6 or 7 breaths, although the magnitude of the response varies significantly from animal to animal. The responses include irregular areas of nonsegmental atelectasis with an increase in airway pressure and a decrease in pulmonary compliance, a decrease in the extractable surfactant from the pulmonary parenchyma, an increase in venous admixture with a decrease in the arterial oxygen pressure, and an increase in extravascular lung water without alveolar flooding or airway fluid (4). In vivo photomicroscopy of the subpleural alveoli in the smoke-damaged lung reveals marked instability of the alveolar wall during tidal respiration, vascular recruitment, and a hyperdynamic circulation. Grossly, the pulmonary parenchymal lesion is nonhomogenous, with areas of dense atelectasis alternating with areas of overexpanded lung; the mechanical stresses at the interface between the pulmonary parenchyma in these two highly divergent states are great and tend to make the pulmonary interstitial pressure more negative, favoring the accumulation of lung water (5). Computed tomography of lungs before and after smoke inhalation confirm that the radiodense areas are atelectasis, because they clear when the images are repeated with the lungs inflated to total lung capacity (6). Histologic sections of these lungs demonstrate severe damage to the mucosa of the airways which remain patent, focal

atelectasis, and accumulation of fluid cuffs around the extra-alveolar vessels.

Lung scans with technetium-labeled diethylenetriamenpenta-acetate acid (^{99m}Tc-DTPA) are used to assess alveolar epithelial permeability (7-8). This radiopharmaceutical is cleared from the lung by absorption from the alveolar surface into the pulmonary capillary circulation; it is rapidly filtered by the kidney so that background levels in extrapulmonary tissues do not accumulate. Following smoke exposure, the DTPA clearance from the dog lung is more rapid, suggesting alveolar epithelial damage (Table I). When the alveolar epithelium is damaged, it becomes more permeable and the fluid balance across it tends to be controlled more by the capillary hydrostatic presure, the interstitial pressure, and alveolar surface tension than it is by osmotic forces; alveolar distension, which may occur in the overdistended portions of the smoke-exposed lung, will also increase the permeability of the alveolar epithelium (9).

TABLE I. Pulmonary Clearance DTPA Data (Mean ± SD)

Smoke Exposure	Half-Time	% Excreted/Min
Control (n = 7)	35 ± 16	2.4 ± 2.3
2-min smoke (n = 5)	11 ± 4*	7.3 ± 2.9*
5-min smoke (n = 8)	6 ± 2*	12.1 ± 3.3*

*$P < 0.05$ vs. control.

Cannulation of a prenodal lung lymphatic allows one to assess lung lymph flow, measure the protein concentration in lung lymph, and compare it to that in plasma. We feel it is important to elevate left atrial pressure in thse lymphatic studies, recruiting the maximum number of vessels possible, so that the study results are not artifactually influenced by significant variations in the vascular surface area exposed to flowing blood. Once the left atrial pressure is increased, pulmonary lymph flow increases following smoke exposure; the concentration of protein in the lung lymph, relative to that in plasma, does not "wash down" or decrease as lymph flow increases, suggesting an increase in the permeability of the pulmonary capillary endothelium (10). When the ratio of protein concentration in airway fluid to that in plasma following smoke exposure is compared to the same ratio seen in surfactant-depleted lungs or lungs exposed to alloxan (100 mg/kg), it seems clear that the permeability defect following smoke exposure varies significantly between animals, is moderate in degree, and is less severe than that seen following alloxan. Similar conclusions are reached when freeze-dried

lung specimens from animals who have had their albumin labeled with Evan's blue prior to smoke exposure or alloxan administration are inspected. The perivascular fluid cuffs are larger, more uniformly distributed, and more densely stained with the albumin marker in the alloxan-treated animals than in the smoke-exposed animals.

When pulmonary surfactant is extracted from 3 g samples of pulmonary parenchyma and tested in the Wilhelmy balance, the surface tension minimum following smoke exposure rises significantly and the normal historesis of the surface tension plotted against the percent of trough area is abolished (4). This observation lead to studies in which pulmonary surfactant was displaced with detergent, and an increase in extravascular lung water was demonstrated without changes in pulmonary microvascular pressure or capillary permeability, confirming the postulated effects of alveolar surface tension on extravascular lung water (11-12). Simply put, in the surfactant-depleted lung, the increase in alveolar surface tension causes adjacent alveoli to collapse away from each other, increasing the negative pressure (vacuum) in the interstitial tissue which causes fluid to collect there. In fact, there does seem to be a relationship between an increase in minimal surface tension of pulmonary extracts and extravascular lung water in both detergent-treated and smoke-exposed lungs (Table II).

TABLE II. Surface Tension and Lung Water in Surfactant-Depleted and Smoke-Exposed Dog Lungs (Mean ± SD)

	Surface Tension Minimum (dynes/cm)	Lung Water (ml H_2O/g dry lung)
Control	8.7 ± 2.5	3.6 ± 0.1
Surfactant-depleted	23.2 ± 0.4	6.1 ± 0.7
Smoke-exposed	26.5 ± 1.7	6.5 ± 1.2

The four acute pulmonary responses to smoke inhalation discussed so far (atelectasis, increase in alveolar epithelial permability, increase in capillary endothelial permeability, and surfactant loss) are all potential contributors to an increase in extravascular lung water. Increasing the permeability of the alveolar capillary membrane has the effect of diminishing the importance of the protein reflection coefficient and the osmotic gradient in the Starling equation, so that lung fluid balance is controlled to a greater extent by the increased fluid filtration coefficient and the pressure gradient in the microvascular portion of the circulation. This

projection seems to be born out in studies in which animals were given a fluid challenge equal to 10% of their body weight of warm Ringer's lactate IV over a 2-h interval following smoke exposure. There were 3 groups of animals, fluid only, smoke only, and both smoke and fluid. The overall fluid balance was the same in both groups of animals receiving the fluid challenge. The extravascular lung water was significantly increased in both groups of animals receiving smoke. Although the increase in extravascular lung water in the smoke-only animals was not statistically different from that in the animals receiving both smoke and fluid, the clinical difference was profound. The animals receiving both smoke and fluid went into florid pulmonary edema, which is never observed in animals receiving smoke alone. The increase in extravascular lung water in the animals receiving fluid only was 2%, smoke only 28%, and both smoke and fluid 42%. This is consistent with similar studies and the observation that alveolar edema does not occur until the extravascular lung water is increased above 35% (13-14). The safety factors which operate so effectively to protect the normal lung from clinical consequences related to increases in extravascular lung water, including an increase in lung lymph flow, an increase in interstitial pressure, and a decrease in the osmotic pressure of interstitial fluid, are no longer able to protect the smoke-damaged lung.

Many factors operate to modulate the fluid balance in the normal lung. Many of the changes detectable within 2 h of smoke exposure operate to make the lung susceptible to fluid accumulation as a consequence of increases in the pulmonary microvascular pressure. This suggests that the smoke-damaged lung is vulnerable to additional stress and has implications for the way in which victims of both smoke inhalation and thermal injuries are resuscitated (15).

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SUMMARY OF CHAPTER II - PULMONARY CHANGES

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PULMONARY DAMAGE from smoke inhalation has probably been recognized since shortly after the discovery of fire. Writings from the first century AD described the torture-execution of prisoners by placing them in cages above green wood fires. However, it was not until the Cocoanut Grove fire in 1942 that the role of smoke inhalation was fully appreciated as a frequent cause of death in burn victims. This appreciation stimulated scientific study of the pathophysiology, diagnosis, and treatment of inhalation injury.

It soon became apparent that heat (except for steam inhalation) seldom caused injury below the pharynx. When inhaled, the heated toxic gases and products of combustion caused severe damage to cell membranes, with the most significant injury occurring in the distal small airways and alveoli. Chemicals commonly contained in smoke include sulfur dioxide, hydrocyanic acid aldehydes, acid anhydrides, and hydrochloric acid. These chemicals cause direct damage and may also react with water to cause corrosive acids and alkalis in the lung. In addition, the oxygen supply is markedly decreased as a result of utilization during burning.

During its 40 years of existence, many members of the US Army Institute of Surgical Research (nee Surgical Research Unit) became interested in this problem and studied the various aspects of inhalation injury. As with many elements of acute burn injury, the pulmonary complications have become both more frequent and more often fatal as other causes of early burn deaths have been controlled or eliminated. Today, pulmonary complications in burn patients are a leading cause of death in both the early and late postburn period and remain one of the unsolved and challenging problems of burn therapy.

These presentations touched upon the most important aspects of inhalation injury. Dr. Moylan discussed the diagnosis of inhalation injury and pointed out the variations in clinical presentation. Despite newer diagnostic techniques, the history of the burn injury and careful physical examination are still important for the diagnosis of the injury. The addition of flexible bronchoscopy and xenon lung scanning has increased the

recognition of pulmonary damage after burn injury. The fiberoptic bronchoscope has the advantage of bedside use, whereas lung scans require transportation of the burn patient for the study and pulmonary function tests demand more patient cooperation than may be possible with the seriously injured. Approximately one-third of all burn patients have been found to have inhalation injury. The early recognition of significant pulmonary damage enables the prompt initiation of therapeutic measures in an attempt to maintain effective oxygenation and prevent subsequent pulmonary insufficiency and infection.

Although early diagnosis allows early treatment, the therapy of inhalation injury is not well established. As in all aspects of burn injury, it is essential for proper treatment of this condition that a basic understanding of the pathophysiology be established. The fact that current treatment of smoke inhalation injury consists of the delivery of humidified oxygen by respiratory ventilator support and mechanical clearing of the respiratory tract indicates that much is to be discovered about the pathophysiology of the condition so that specific and effective therapy can be developed.

The other presentations discussed laboratory animal investigations of pulmonary injury. Drs. Herndon and Shimazu have both developed a sheep model to investigate the pathogenesis of inhalation injury. Dr. Herndon concluded that smoke inhalation causes microvascular permeability changes. He also pointed out that lung lymph flow is dependent upon the duration of smoke inhalation which also stimulates sequestration of neutrophils, releasing proteolytic enzymes and free radicals that cause microvascular injury to bronchial and lung parenchyma. The resulting edema formation decreases pulmonary function and arterial oxygenation. Dr. Herndon noted that microvascular permeability damage is attenuated with heparin, polymorphonuclear leukocytes are depleted by nitrogen mustard, and that dimethyl sulfoxide and the proteolytic enzyme inhibitor, gabexate mesilate, attenuated the edema seen after inhalation injury. The model described should be useful in evaluating possible mediators of pulmonary dysfunction following smoke inhalation injury.

Dr. Shimazu used a similar animal model to measure severity and time-related alterations in cardiopulmonary indices, including ventilation-perfusion ratios, and found them to be similar. Progressive hypoxia, lower lung compliance, and higher airway resistance were noted as time passed and as injury increased. There was progressive distortion of the perfusion pattern with development of very low ventilation-perfusion compartments which explained the etiology of the hypoxia. Some animals developed a substantial increase in shunt flow which contributed to progression of hypoxia.

A dog animal model was employed by Dr. Clark to investigate lung fluid balance after inhalation injury. He reported that animals receiving both smoke injury and fluid therapy went into severe pulmonary edema which was not observed in animals receiving smoke alone. Extravascular lung water was also markedly increased in the group receiving both smoke and fluid (42%), as opposed to those receiving smoke alone (28%). Many factors which normally protect the lung from increased lung water are no longer operative in the smoke-damaged animal. The changes which occur after inhalation injury make the lung susceptible to fluid accumulation and have implications for the methods of resuscitation in patients with both thermal and pulmonary injury.

These presentations are indicative of the studies being performed by US Army Institute of Surgical Research alumni and other investigators to elucidate the pathogenesis of inhalation injury so that effective treatment modalities can be developed to decrease the present high mortality and morbidity from this aspect of burn injury. A better understanding of the pathophysiology of this injury will eventually allow improved fluid therapy and medical management of these patients in addition to the oxygen delivery and tracheal toilet treatments presently available but frequently inadequate.

CHAPTER III - WOUND CARE

ELECTRO-OPTIC AND ACOUSTICAL ASSESSMENT OF BURN DEPTH

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THIS RESEARCH program successfully developed a real-time video imaging system (the Imaging Burn Depth Indicator (IBDI)) which can discriminate between areas of burn wounds expected to heal in 3 wk or less from the day of injury and those areas not expected to heal in that time period. The analysis can be performed on or about the third day postburn on debrided burn wounds. Early evaluation of burn healing probability is a crucial factor in the decision to tangentially excise the burn wound.

The IBDI measures the reflectivity of the burn wound in the red, green, and near-infrared wavelength bands and this data is then correlated with burn wound healing probability. This instrument uses an algorithm established in an earlier study to translate this optical data into burn healing probabilities (1).

The IBDI produces two types of images, i.e., a true-color image of the burn and a false-color image of the burn. The false-color image consists of up to 4 colors, each of which indicates a distinct range of probability that the area of the burn so colored will heal within 21 days.

During the first year of the study, the instrument was designed and fabricated. During the second year, the system was tested in a clinical setting at the Burn Center at Harborview Medical Center (Seattle, WA). Over 100 burn wound sites were studied. Burn sites were evaluated on the third postburn day by our instrument and by the attending physician. Of those sites predicted by the instrument to heal in fewer than 21 days, the IBDI was correct 91% of the time. Of those sites predicted to not heal within 21 days, the IBDI was correct 85% of the time. By comparison, the predictions of burn surgeons supervising the care of these patients were 76% correct on burns predicted to heal within 21 days and 67% correct on burns predicted to not heal within 21 days.

In our clinical tests, the instrument was more accurate in predicting burn wound healing than the participating burn surgeons. Yet, the IBDI can be operated by a person of average intelligence with no specialized burn training. Thus, with the IBDI, evaluation of burn victims and selection of treatment modalities may be accomplished in the absence of qualified burn specialists.

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THE ROLE OF ENZYMES IN BURN CARE

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HISTORY

After thermal injury is inflicted on the skin, an interface is created between damaged tissue and deeper viable tissue. Eschar represents that portion of necrotic skin consisting primarily of denatured collagen, elastin, and small amounts of other proteins. Krizek et al (1) observed that eschar separation occurred spontaneously about 10-14 days postburn when topical antibiotics were not used. Due to its avascularity and abundance of protein, eschar acted as a pabulum for infection, allowing bacterial proliferation in the wound. Collagenase, elaborated by bacteria colonizing the burn wound, proved to be the catalyst responsible for early lysis and separation of necrotic tissue.

Vistnes and Hogg (2) performed serial biopsies on patients who had sustained full-thickness burns and were treated with topical gentamicin. Conclusions drawn from this study indicated that collagen fibrils were readily thermocoagulated by heat, whereas elastic fibers were relatively resistant to burning. As long as 3 wk postinjury, elastic fibrils appeared normal histologically and maintained bridging fibers across the zone between necrotic and viable tissue. Eschar separation occurred between 4 and 5 wk postburn, at which time elastic fibers became fragmented. From this discussion, it was apparent that elastin hindered the early detachment of eschar. Furthermore, the topical antimicrobial agents applied to the eschar suppressed bacterial growth, resulting in a retardation of spontaneous eschar separation, which in turn delayed final closure of the wound.

With the availability of sophisticated resuscitative fluid regimens, sepsis, not hypovolemic vascular collapse, presents the greatest threat to life. Thus, a safe method to shorten the time of eschar separation, expedite wound closure, and decrease the possibility of sepsis would dramatically affect both morbidity and mortality associated with burns. For example, patients with extensive burns who cannot tolerate the physiologic stress of surgical debridement would benefit from topical application of an agent that enhances eschar removal and decreases the time required for spontaneous reepithelialization or first skin grafting. Enzymatic debridement presents an appealing addition to the armamentarium of burn wound care.

Use of enzymes for wound debridement is merely an extension of the natural biologic process of healing, but the search for suitable enzymes has been disappointing. The unsuitable agents were either toxic to adjacent viable tissue, not specific for the eschar as substrate, or too slow in proteolysis of eschar to shorten the time until the first skin grafting. During the last quarter-century, several enzymes from plant, animal, and bacterial sources have been proposed as effective debriding catalysts with disappointing results.

Papain. In 1943, investigators reported the use of papain, an extract from the papaya plant which possesses proteolytic activity, as a potentially effective enzyme for debridement. However, Beard et al (3) found that "papain in the absence of activators and denaturants was relatively inactive." Further studies by Burke and Golden (4) incorporated urea and chlorophyll with papain to enhance proteolytic activity in debridement of decubitus ulcers, burns, and other necrotizing lesions while diminishing toxicity to adjacent tissue. Papain research revealed that proteolytic activity exceeded the limits of damaged tissue, though eschar removal remained patchy, indicating that thermocoagulated skin was not an optimal substrate for papain.

Streptococcal Enzymes. Later, Connell and Rousselot (5) proposed the use of streptococcal enzymes as possible debriding agents. Streptokinase, a fibrinolytic substance known to catalyze the transformation of plasminogen to plasmin, and streptodornase, which lysed deoxyribose nuclear protein, were studied. As a result, three important parameters for the implementation of enzymatic debridement were illustrated. First, proper substrates must be present in the evolving tissue slough for enzymes to effect atraumatic separation of necrotic tissue. Second, since thermocoagulated tissue is composed of a complex mixture of proteins, an enzyme with broad-spectrum activity is required. Third, streptokinase and streptodornase demonstrate no activity against collagenous protein, epithelial cells, or fibroblasts, thereby rendering them ineffective in the treatment of burns.

Continued studies were performed to test other proteolytic enzymes (trypsin and chymotrypsin) for debriding purposes. Unfortunately, these digestive enzymes lack the substrate specificity needed for an effective debriding agent. Analysis of the repeated failures of different enzyme systems contributed to the elucidation of the criteria necessary for future success, i.e., requirements of enzyme-substrate specificity must be satisfied, optimum enzyme activity must proceed at physiologic temperature and pH, and heavy metals or other agents that denature enzymes must be absent from the wound environment.

Collagenases fulfill the requirement of substrate specificity and are active at physiologic pH. Enzymes

investigated with these properties include isolates of Clostridium histolyticum. Hummel et al (6) demonstrated satisfactory debridement of small full-thickness burns by clostridial collagenase, effecting complete eschar separation after 24-96 h of therapy. Best results were achieved within the first 48 h postinjury when the eschar was soft and moist. Mature, leathery eschar was relatively resistant to enzyme penetration. A serious complication of enzymatic debridement was sepsis, which occurred in 6 of 11 the patients studied. Subsequent concomitant use of appropriate antibiotics and/or topical antimicrobials was required to maintain negative blood cultures.

The investigation of clostridial collagenases made it apparent that a more efficacious enzyme was needed for the debridement of burn wounds. Enzymes produced by other Gram-negative bacilli such as Escherichi coli, Pseudomonas aeruginosa, and Proteus mirabilis were tested. All three enzymes proved disappointing due to incomplete debridement and associated sepsis.

Nonspecific proteases were next isolated to solve the problems of enzymatic debridement. Sutilains, a natural protease obtained from Bacillus subtilis, catalyzed proteolysis and exhibted optimal activity at a pH range of 6.0-7.5 for protein substrate at body temperature. Lytic activity was demonstrated for a wide variety of proteins found in eschar, including limited activity toward collagen.

Garrett (7) studied the use of sutilains in 240 patients with good results. Up to 40% of the eschar was digested in 24 h and reapplication of the ointment for another 48 h resulted in 80% debridement of full-thickness burns. Eschar still adherent after sutilains application was softened, thus facilitating subsequent mechnical removal. Analysis of the degradation products of eschar revealed acid-soluble peptide, amino acid fragments, and some hydroxyproline, suggesting collagenolytic activity. It was apparent from these results that effective debridement could be accomplished without significant risk of sepsis. Sutilains penetrated the entire eschar and showed little toxicity to viable tissue. Soft, moist eschar was more suitable for enzymatic debridement than the matuie, desiccated crust that develops after the first 2 days postburn.

Garrett concluded that "When debridement was initiated within 48 h after injury, it was often possible to begin resurfacing the third degree areas as early as the second week postburn." Prompt application of sutilains, intimate enzyme-substrate contact in a moist environment at body temperature, and the concomitant use of topical antimicrobials were critical factors leading to effective and safe debridement of the burn wound. Further studies by Pennisi et al (8) substantiated successful clinical treatment with sutilains.

Bromelain, an extract of the pineapple stem, exhibits broad-spectrum protease activity against native protein. Its potential as an effective debriding agent has been recognized by several investigators, although purification techniques have slowed the ability to isolate the active derivatives. Polyacrylamide gel electrophoresis reveals that bromelain contains at least 4 separate fractions exhibiting broad-spectrum protease activity (9). The enzyme is active over a wide pH range and demonstrates maximum activity at a low concentration.

Silverstein et al (9), utilizing a controlled in vitro environment, compared the efficacy of hydrolysis by sutilains, bromelain, and clostridial collagenase on a variety of substrates, including viable and denatured collagen, pigskin, and human eschar. Both peptide hydrolysis and hydroxyproline release were measured to quantitate nonspecific proteolysis and collagenolysis, respectively, for each enzyme system. These results (shown at Tables I and II) led to the conclusion that sutilains was most effective in catalyzing nonspecific protein hydrolysis, collagenase demonstrated the most effective hydrolysis of pure collagen in its natural (microcystalline) and denatured (gelatin) forms as measured by hydroxyproline release, sutilains activity in both pigskin and human eschar was greater than collagenase and bromelain, and bromelain, in the concentrations studied, consistently showed less effective hydrolysis of all substrates tested than sutilains.

This study was significant because it quantitated proteolytic activity of enzymatic debriding agents using substrates encountered in the natural process of eschar separation at controlled temperature and pH. Sutilains displayed effective debridement because of its activity on both nonspecific proteins and collagen found in the burn eschar. As a result of the studies cited previously, sutilains is the most frequently used enzyme in burn centers today.

CLINICAL TECHNIQUE

Several considerations must be observed to maximize the safety and success of enzymatic treatment, including assessment of the patient's general medical status, area and extent of burn, time postburn, dressing techniques, and concomitant topical chemotherapy. Sutilains ointment is indicated for any type of burn, including those caused by flame, scald, chemicals, or electricity. Enzymatic agents have not been advised for use over major blood vessels, tendons, ligaments, bone, viable tissue, or on the face, due to possible irritation of the conjunctiva. They are also contraindicated during pregnancy (10).

Enzymatic debridement should be used with caution on patients who are hemodynamically unstable, since the use of enzymes may result in excessive fluid loss through the area

TABLE I. Rate of Protein Hydrolysis by Protease Action in Various Substances*

	Specific Activity (nmoles leucine/min/mg enzyme)		
	Sutilains	Bromelain	Collagenase
Bovine serum albumin	267	117	2
Pigskin gelatin	62	53	75
Microcrystalline collagen	9	5	77
Split-thickness pigskin	59	49	30
Human eschar	77	39	61
Boiled split-thickness pigskin	79	45	51

*From Silverstein P et al (15). Table used with permission.

TABLE II. Rate of Peptide Hydrolysis vs. Hydroxyproline Released During Enzymatic Treatment of Split-Thickness Pigskin

Enzyme (mg/cm^2)	Protease Activity (nmoles leucine/ml/min)	Hydroxyproline Released (nmoles/ml/min)
Sutilains:		
0.625	37	1.5
0.940	43	1.9
1.25	59	1.6
Bromelain:		
0.625	23	1.2
0.940	29	1.6
1.25	38	2.1
Collagenase:		
0.625	13	8.6
0.940	16	9.3
1.25	19	12.2

*From Silverstein P et al (15). Table used with permission.

being treated, thereby potentiating hypovolemia. It is therefore advisable to monitor urinary output and blood pressure closely. Only 20% or less of the total body surface should be treated with enzymes at any one time (10). The limitation of body surface area not only diminishes the aggravation of fluid imbalance but also helps reduce the potential for rapid invasion of bacteria into subjacent tissue.

Use of enzymes has been observed to cause minor bleeding as the eschar separates from the viable wound interface. Patients also complain of a "burning" sensation for 20-60 min after application. Transient minor temperature elevation may follow dressing changes. Cellulitis has also been noted around the periphery of the burn wound. Prolonged treatment with enzymes can result in contact dermatitis and/or maceration of adjacent unburned skin. No systemic toxicity has been reported (10).

Enzymatic debridement is usually successful when started within the first 48 h of burn injury up to 7 days postburn and continued for 5-10 days. Surgical escharotomies may be unnecessary when enzymes are applied to circumferential burns of the extremities due to softening and rapid digestion of the devitalized tissue and subsequent decompression (11). The

advantages of enzymatic "escharotomy" include maintenance of range-of-motion exercises during dressing changes, noninterference with splinting, preservation of subdermal vasculature, and prevention of the need for surgical intervention.

Grant (12) reported that the rapid action of sutilains permits successful grafting of deep partial-thickness hand wounds within the first 24 h postburn, thereby expediting wound closure and allowing early mobilization and restoration of function with minimal contracture formation. Sutilains' specific action on nonviable tissue is helpful in differentiating deep partial-thickness from full-thickness burns within the first 48 h postinjury. Sutilains will debride down to subcutaneous fat beneath a full-thickness burn; it will not convert a partial-thickness burn to a full-thickness loss. Nevertheless, conversion of the burn could occur due to bacterial invasion secondary to poor dressing techniques.

The age of the wound at the onset of treatment is important in predicting the efficacy of enzymatic treatment. Proteolysis progresses most effectively on the acute, moist wound. Water required for the hydrolytic activity of enzymes can be provided by wet saline dressings or the hydrated bases of antimicrobial agents, e.g., silver sulfadiazine, mafenide acetate. The technique of dressing application is of utmost importance for optimal enzymatic debridement and prevention of infection.

Careful selection of topical antimicrobials is imperative and care must be taken to avoid the inadvertent use of enzyme-denaturing agents containing heavy metals incorporated in many skin detergents, soaps, and antiseptics.

Sterile technique with cap, mask, and gloves should be practiced when applying enzymatic dressings. After the wound is washed with a compatible bactericidal cleanser in the hydrotherapy tank, shower, or at the bedside, the enzyme may be applied in one thin layer, either directly to the wound or after impregnation of a gauze-type dressing. If incompatible cleansing agents are used, the wounds must be rinsed thoroughly with water before application of the enzyme. Enzymatic penetration of full-thickness eschar may be enhanced by cross-hatching with a sterile scalpel blade. Enzyme contact with unburned skin and eyes should be avoided because of possible irritation. Following application of the enzyme and one layer of fine-mesh gauze, a topical antimicrobial agent such as silver sulfadiazine, triple antibiotic cream/ointment/solution, or mafenide acetate is added. The wound is then covered with a bulky wet dressing and immobilized with expandable mesh gauze.

Since enzymes by themselves provide no antimicrobial protection, it is mandatory to incorporate a compatible topical antimicrobial agent to suppress bacterial proliferation.

Considering the rate of enzyme absorption into the eschar, dressings are ideally changed every 4-6 h. Enzymatic reactions are sensitive to environmental temperature; therefore, because the enzymes are themselves protein molecules, they should be stored in a refrigerator to slow autodigestion and prolong potency.

When used properly, enzymatic agents are an effective adjunct to debridement of the burn wound, leading to early wound closure and decreased length of stay and cost of hospitalization. Average date of first graft is usually between 2 and 14 days postburn and average date of discharge is 7-14 days earlier than in patients treated without enzyme therapy. The application of enzymatic agents is by no means a panacea for burn wound care. However, the alternative methods available for the combined use of enzymes with different topical antimicrobials can provide the flexibility needed to treat the varied requirements of individual patients.

FUTURE DEVELOPMENT OF ENZYMES

Adjuvant enzymes, used in conjunction with nonspecific proteases to enhance proteolysis of elastin fibers, and new dosage forms of existing enzymes may provide a more effective mechanism for early debridement. Elastase is a proteolytic enzyme with specific activity on elastin fibils. The potential value of facilitating eschar separation with elastase was hypothesized, since elastin binds the interface between necrotic and viable tissue for up to 4 wk (2). Research on the enzymatic effects of elastase on human eschar has been limited. Silverstein et al (13) addressed this question and concluded that elastase did not accelerate the rate of proteolysis of burn eschar *in vitro*, possibly because of its requirement for an alkaline pH not normally found in the burn wound.

The combined effects of sutilains with collagenase would seem to provide complementary interaction, one possesses nonspecific protease activity and the other specific collagenolytic activity. *In vitro* studies performed by Silverstein et al (9) revealed no enhancement of nonspecific proteolytic activities. In fact, ". . .the mean rate of hydrolysis by the combination of enzymes was only slightly greater than the rates of the individual enzymes measured separately and added together" (see Table III).

Sequential application of sutilains followed by collagenase did show an increase in the amount of hydroxyproline released during the second incubation period with collagenase (Fig 1). This specific sequence of enzymes may accelerate hydrolysis of human eschar when nonspecific proteases are employed to pretreat the wound and expose the collagen in the deeper dermal layers to collagenase. Subsequent application of collagenase would therefore allow maximum substrate contact, resulting in a more effective hydrolysis of collagen. Since collagenolysis is

TABLE III. Effect of Combination of Enzymes on Protease Activity During Enzymatic Treatment of Split-Thickness Pigskin

Sutilains A	Collagenase B	Sutilains + Collagenase C	Calculated Activity A + B
5 mg	10 mg	5 + 10 mg	
29	6	39	35
7.5 mg	7.5 mg	7.5 + 7.5 mg	
34	5	44	39
10 mg	5 mg	10 + 5 mg	
50	3	56	53

*From Silverstein P et al (15). Table used with permission.

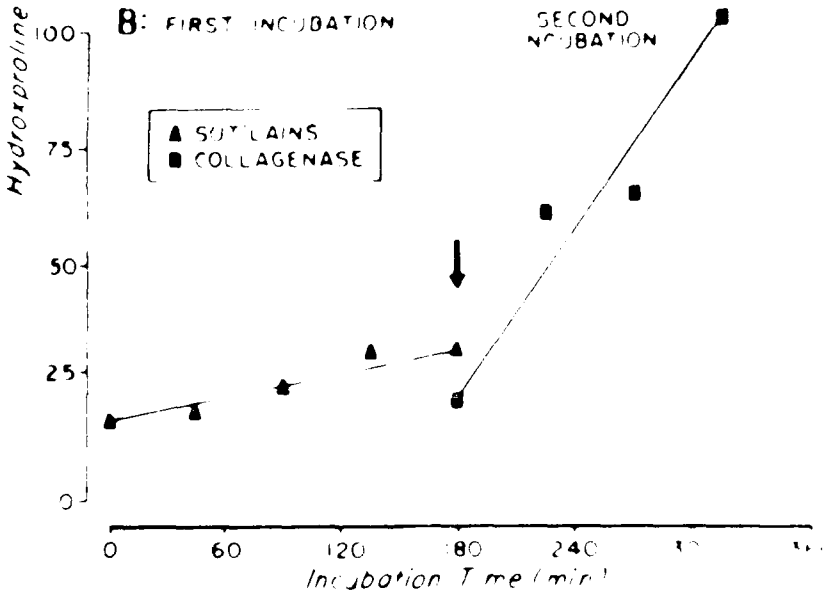

FIGURE 1. Sequential application of sutilains and collagenase.

critical to eschar separation, combinations or sequences of enzymes which increase hydrolysis may provide a method of debridement superior to the use of a single agent.

Enzymatic debridement with bromelain in the laboratory resulted in effective and consistent removal of eschar without damaging adjacent viable tissue. Levine et al (14) reported that bromelain applied to full-thickness burns in 3-wk-old pigs

demonstrated unifrom debridement of burn eschar within 2 h after its application. Interestingly, these investigations indicated that the debridement was achieved by "dissection," whereby the eschar was separated in one layer instead of layer by layer. The significance of this action of bromelain has yet to be elucidated.

Levine et al also investigated the success of skin graft application to porcine burn wounds debrided with bromelain. The wound bed appeared clean before grafting, but the results after skin grafting were disappointing. At 10 days postgraft, transplanted tissue became necrotic. Subsequent investigations using a more purified enzyme produced debrided wounds in swine that successfully accepted a skin graft with good results. Attempts to purify the active fractions of the bromelain complex and determine the concentrations for efficacious and safe utilization in an aqueous-gel vehicle on human patients are currently under way.

The future of enzymatic debridement will depend on the production of new dosage forms of specific collagenases and nonspecific proteases that safety digest eschar within 24-48 h, leaving a wound bed capable of providing nidation for a skin graft. Sequential application of various enzymes to hydrolyze thermocoagulated skin may provide the key to widespread use of nonsurgical debridement in coming years. Ideally, these agents will be inexpensive, painless, easy to apply, free of allergic side effects, and nontoxic to viable tissue within the wound or unburned skin at its periphery.

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EFFECTS OF LOW VOLTAGE DIRECT CURRENT ON THE BURN WOUND

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THE EXPERIMENTAL burn treatment introduced here utilizes a silver-nylon dressing which, when used as a direct current anode, produces both antimicrobial and wound healing effects. We have used two animal species for these studies. Antimicrobial activity was investigated using the Walker-Mason burned rat model (1). Wound healing activity was investigated in guinea pigs.

Silver-Nylon Cloth. Silver-nylon cloth (Style A-2589-5, Swift Textile Metalizing Corporation, Hartford, CT) is a soft, flexible material consisting of nylon coated with metallic silver (SN). It contains 3 g Ag/m^2. This cloth served as a flexible anodal electrical contact.

Auto-Adjusting Direct Current Generator. The generator provided a constant amperage flow in the range of 0.4 to 100 μA within a limited range of the voltage fluctuation. This is a simple voltage-controlled current source suitable for delivering 8-16 isolated animals with voltage over-range safety cutoff set at 15 V. When direct current (DC) below 100 μA was applied to animal using a SN anode, the highest voltage observed was 0.86 V (2).

Experimental Animal Models. Three types of animal models were designed for different study purposes as follows:

Rat Model for Testing Antimicrobial Effect Investigation. Male Sprague-Dawley rats weighing 250 $\pm$ 25 g with 20% full-thickness dorsal scald burns using the Walker-Mason method were used as experimental animals. For infection, the wounds were topically inoculated with *Pseudomonas aeruginosa* (Strain 1244) at 100,000,000 organisms per rat (=1,500,000/cm^2) or *Proteus mirabilis* (Strain 7708234) at 100,000 organisms per rat (=1,500/cm^2) 1 h after scalding. At 4 and 24 h postburn (PB), SN or uncoated nylon dressing as an anode was sutured over the infected burn wound. An electrical circuit was established by placing a 2.5 cm x 1.0 mm silver needle as a cathode under the burn wound at the subpanniculus carnosus space through the dorsal neck skin. In this configuration, polarity of the dressing and needle could be established by switching electrode connections. After suturing the dressing, 3 layers of gauze and a layer of sponge with a small polyethylene irrigation tube attached underneath was placed on the dressing. To prevent the rat from gnawing

the electrode wires and irrigation tube, the wires and tube were passed through a hole cut in a wooden tongue blade and surrounded by a 4-in mesh wire insulator. The blade was then sutured to the animal's back over the dressings. The gauze and blade were fixed with a meshed flexible tubular bandage. The animals were thus free to move inside their cages, but were denied access to the wires and tubes. The gauze was moistened with 3-5 ml saline through the irrigation tube; this was repeated 2-3 times per day when DC was applied. During treatment, different test groups received DC from 0.4 to 100 μA, which was constantly and continuously applied over the 60 cm^2 burn wound for 5 days. Antimicrobial effects were compared to infected animals without further treatment and to animals treated with a known therapeutic agent, silver sulfadiazine. Mortality was recorded at 21 days PB. Results of SN anodal treatment of Pseudomonas aeruginosa and Proteus mirabilis-infected rats are presented in Tables I and II, respectively. As can be seen, SN used as an anode was effective in treating both infecting organisms.

TABLE I. Mortality of Pseudomonas aeruginosa Control/ Treatment Groups (n = 20/Group)

Group Number	Description	Mortality (%)
Control Group		
1	Burned + infected + UN	80
Treatment Groups		
2	Burned + infected + SN anode (40 μA/4 h)	5
3	Burned + infected + SN anode (4 μA/4 h)	0
4	Burned + infected + SN anode (0.4 μA/4 h)	0
5	Burned + infected + SN	0

( ) indicates time postinfection that treatment was begun; UN, uncoated nylon; SN, silver-nylon.

Guinea Pig Model for Burn Wound Healing Effects. The only treatment difference between this guinea pig model and the previous rat model is that in the guinea pig model, a piece of SN cloth fixed on the anterior chest and abdominal wall as a cathode replaces the inserted cathodal silver needle used in the rat model. Between the cathodal SN and skin, there was a layer of EKG conductive gel. We used this guinea pig model to

TABLE II. Mortality of *Proteus mirabilis* Control/Treatment Groups (n = 20/Group)

Group Number	Description	Mortality (%)
Control Group		
1	Burned + infected + UN	75
Treatment Groups		
2	Burned + infected + SN anode (4 μA/2 h)	10
3	Burned + infected + SN anode (40 μA/2 h)	10
4	Burned + infected + SN	0

( ) indicates time postinfection that treatment was begun; UN, uncoated nylon; SN, silver-nylon.

examine the healing effects of weak DC on partial-thickness and full-thickness scald wounds.

Partial-thickness wounds were inflicted by a 10-sec exposure of depilated dorsal skin to 78-80°C hot water. Full-thickness wounds were inflicted by a 10-sec exposure to 100°C boiling water. The burn wound covered 18% of the total body surface area. In the full-thickness scald wound healing study, PB cooling treatment was added in 2 study groups by a 15-min application with a plastic bag containing 4-8°C cold water on the burned wound immediately after scalding. During treatment, 40 μA DC was constantly and continuously applied for the first 2 days PB and 20 μA from the third to the fifth PB days. Treatment and control wounds were compared histologically and grossly at selected times PB. The results of partial-thickness scald wound healing studies showed:

1. Anodal treatment reversed circulatory stasis by 48 h PB. Control animals required 96 h.

2. Anodal treatment maintained the viability of >90% of the dermis and the majority of hair follicles. Control animals lost 25% of the dermis and only a few of the deepest hair follicles survived.

3. At 3 months, treated animals showed minimal wound contracture and abundant hair growth. Control animals showed moderate to severe wound contracture and hair loss.

Full-Thickness Scald Wound Healing Studies. The results demonstrated:

1. Anodal treatment with immediate cooling resulted in survival of 50% of the dermis. Anodal treatment alone resulted in 30% survival of the dermis. Control animal wounds were full-thickness sloughed to below the panniculus carnosus muscle.

2. Anodal treatment resulted in reepithelization from deep hair follicles by 3-4 wk.

3. At 4 months, all treated wounds showed moderate contracture (30-40%). Control animals healed by complete contracture. Anodal treatment with immediate cooling resulted in moderate hair survival.

CONCLUSIONS

SN anodal dressing with weak DC is an effective antimicrobial agent in the burn rat models of Pseudomonas aeruginosa and Proteus mirabilis burn wound sepsis. Anodal current dressings accelerate healing time and quality of partial-thickness wounds and grafts. Under the condition of these studies, SN dressings rescue deep scald injuries from conversion to full-thickness injuries.

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BIOLOGIC DRESSINGS AND SKIN SUBSTITUTES

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THE PURPOSE of this study was to develop a synthetic temporary skin substitute. Empirical laboratory studies resulted in the development of a temporary skin substitute consisting of a Teflon^{R} membrane laminated to a nylon matrix.

Studies using this temporary skin substitute in rats demonstrated favorable properties relating to both membrane function and wound closure. The dressing conformed well to denuded wounds and was rapidly invaded by fibroblastic and vascular ingrowth. The dressing limited microbial colonization of contaminated wounds and, like allograft and xenograft, prevented death from invasive infection which occurred in contaminated control animals where no wound cover was used. In terms of membrane function, the dressing promoted survival when used to cover lethal excisions of 60% of the total body surface area of a rat.

A clinical evaluation of this dressing was performed in a formal study on modest-sized areas of burn wound granulation tissue. Its effect on wound appearance and surface quantitative microbiology was compared to that of human cutaneous allograft, porcine cutaneous xenograft, and coarse-mesh gauze during a 48-h treatment period. A significant decrease in wound bacterial counts was observed exclusively with allograft treatment when the allograft was well adherent. No other form of treatment significantly altered bacterial colonization of the wound. On wounds where allograft was adherent, there were no significant difference in wound appearance among areas treated with allograft, coarse-mesh gauze, or the synthetic dressing. Areas treated with porcine cutaneous xenograft appeared worst.

For wounds on which cutaneous allograft was nonadherent, gauze and the synthetic dressing resulted in the best appearance, with cutaneous allograft followed by porcine cutaneous xenograt appearing significantly worse. For wounds on which allograft was nonadherent, no significant change in bacterial colonization was observed following coverage with any of the four treatment modalities.

This study reinforces the advantage of coverage with cutaneous allograft for graftable wounds. However, it provides no evidence to support the use of viable biologic dressings to prepare wounds for graft acceptance. Such wounds are best

treated with either the synthetic dressing or coarse-mesh gauze.

A clinical trial of the dressing suggested that it could be used conveniently and safely. It conformed well to irregulator surfaces and permitted motion of joints covered with the dressing. It did not fragment into the wound. If separation occurred under the dressing, it was easily recognized. The principal advantage of this dressing was to provide membrane function as well as debridement for denuded wounds, which involves the removal of a small amount of surface debris before grafting can be achieved. For graftable wounds, it ranked second to human cadaver allograft as a temporary skin substitute.

MANAGEMENT OF PATIENTS WITH MASSIVE BURNS

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The major determinants of survival for patients with burn injury have been age, burn size, inhalation injury, and infection. In light of the current enthusiasm for early excision of the burn wound, we reviewed our results of care in patients with massive burn injury.

Seven hundred and seventy-seven patients were admitted to the Institute between 1 January 1983 and 31 December 1986. Six hundred and thirty-seven patients were male, reflecting the predominance of male burn-injured patients. The ages for this patient group ranged from 6 wk to 97 yr. Sixty-three percent of these patients were admitted to the Institute within 24 h of injury.

To illustrate the overall improvement in survival at our Institute, we generated a computer plot which subtracts the death rate as related to burn size and age in the years 1980-84 from the death rate in the years 1950-63. The % improvement in survival is reflected on the vertical axis in terms of % difference as related to burn size and age (Fig 1).

Two hundred and eighty-three of these 777 patients (36.4%) met the arbitrary defirition of massive burn injury, established for this review as burns of 30% of the total body surface area or greater. There were 251 males and 32 females whose ages ranged from 6 wks to 93 yr. The average burn size of this subset of 283 patients was 52.5% of the total body surface area, with an average 31.5% full-thickness or 3° injury. A diagnosis of inhalation injury, established by a combination of history, physical examination, fiberoptic bronchoscopy, and xenon 133 ventilation-perfusion lung scan, was made in 153 (54%) of these patients.

One hundred and sixty-six patients (58.7%) survived. The average age of surviving patients was 27 yr and the average burn size was 45.3% of the total body surface area with 21.6% full-thickness burn. Inhalation injury was present in 57 patients (34%). Excision of the burn wound was necessary in 152 of these 166 patients and the average time of the first excision was 12.6 days postinjury, with a range from the second to the 31st day postinjury. Those patients who were excised later in their burn course were patients with extensive injury and minimal full-thickness burn.

One hundred and seventeen of these 283 patients (41.3%) died. The average age of patients who died was 43.1 yr and the

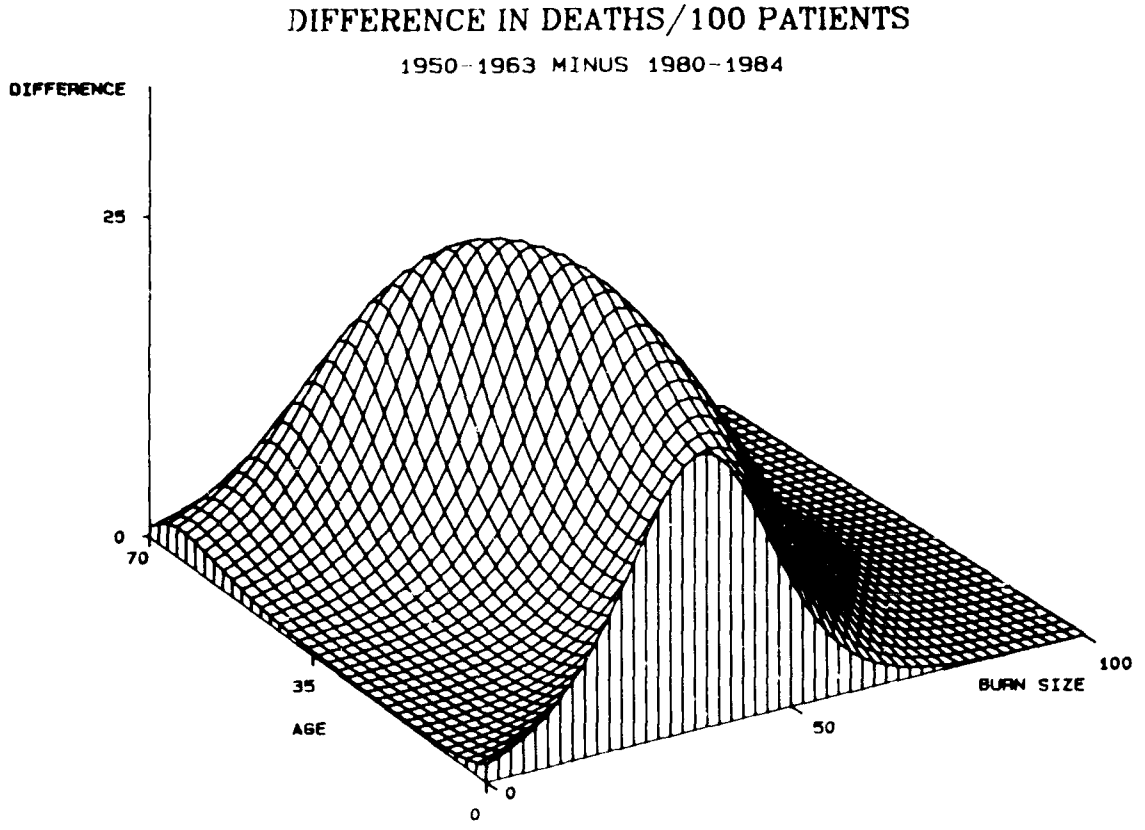

FIGURE 1. Computer plot showing difference in deaths for 100 patients during 1950-63 and 1980-84 at the US Army Institute of Surgical Research.

average burn size was 63% of the total body surface area with an average of 45.3% full-thickness injury. Ninety-six of these patients (82%) had inhalation injury. Excision of the burn wound was possible in only 52 of the nonsurviving patients (44%). The average time of first excision in these 52 patients who were able to be taken to the operating room was 11.2 days, not different from surviving patients. The range of the first postinjury day of excision was from as early as 2 days to as late as 28 days.

In order to accurately reflect our timing of excision, we identified those patients admitted within 48 h of injury who had >9% full-thickness injury, thus eliminating from consideration patients admitted late or those who had extensive partial-thickness injury and only required minor autograft procedures to complete wound closure. There were 88 survivors and 89 nonsurvivors. The average postburn day of the first excision had progressively decreased from 1983-86.

Forty-five patients with a total burn size exceeding 30% and full-thickness burn size exceeding 9% were admitted within 48 h of injury in 1986; 22 survived. The average age of surviving patients was 24.3 yr and the average burn size was 46.3% with an average full-thickness injury of 28.2%. Forty-six percent had inhalation injury. The average time of first excision was 8 days postinjury with a range from the fourth to the 18th postinjury day.

Twenty-three patients died. The average age of patients who died was 37 yr and the average burn size was 68.4% with an average full-thickness injury of 46.2%. Seventy-four percent had inhalation injury. Excision of the burn wound was possible in only 12 patients. The average time of first excision in these 12 patients who were able to be taken to the operating room was 6.8 days and ranged from the fourth to the 10th postburn day.

Infection was the cause of death in 84 of these 117 patients (72%) and pneumonia was the single most common infection in 66 of the 117 patients who died. Burn wound infection was histologically identified as the cause of death in 15 of the 117 nonsurviving patients. Bacterial infection was present in only 1 patient, fungal infection in 12 patients, and viral infection in 2 patients. Causes of death other than infection were identified in 33 patients, of which 14 were inhalation injury, 10 acute myocardial causes, 7 miscellaneous causes to include phenol toxicity, tracheoesophageal fistula, and cerebral edema secondary to hypoxia, and finally, 2 with pulmonary embolii.

As previously from this Institute (1), inahlation injury alone is associated with a maximum added mortality of 20% as related to burn size and age. Pneumonia is associated with a maximum added mortality of 40% as related to burn size and age. The combination of inhalation injury and pneumonia, which are significant independent and additive comorbid factors, is associated with a maximum added mortality of 60% as related to burn size and age.

The effect of age and burn size is demonstrated at Figure 2, where the vertical axis represents age groups of 0-15, 16-40, 41-60, and >60 years of age and the horizontal axis represents burns of 30-50%, 51-70%, and 71-100% of the total body surface area. The number above the diagonal line represents those patients who survived and the number below the diagonal line represents those who died in each category. Mortality increases from the upper left to the lower right, demonstrating the influence of burn size and age in these 283 patients (Fig 2).

Various investigators have reported improved survival and, conversely, no survival advantage in patients who underwent early excision. In examining three such reports, one claimed

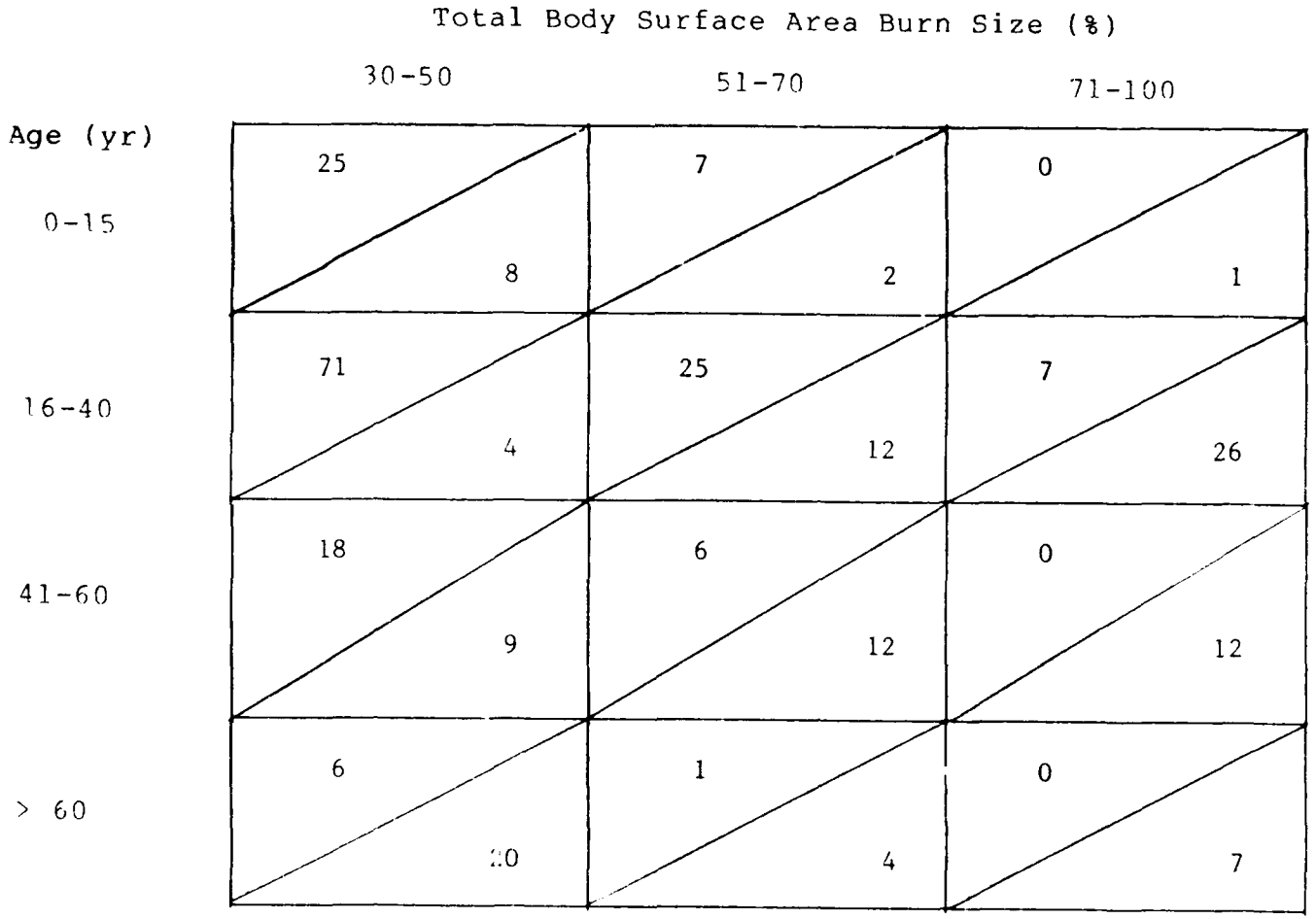

FIGURE 2. Effect of age and burn size on mortality.

improved survival with excision; however, there were no controls, the study spanned a period of approximatley 45 years in which there has been a general improvement in overall survival not necessarily attributable to excision, and the majority of patients in this report had burns of 20% of the total body surface area or less (2). Another investigator reported on the results of early excision in patients with burns of 20-40% of the total body surface area and identified no survival advantage (3). A third investigator reported a controlled study in which there was no survival advantage of early excision of burns that exceeded 15% of the total body surface area (4).

The unquestioned indications for excision of the burn wound include full-thickness burns of limited extent, deep dermal dorsal hand burns when the total burn is of limited extent, the debridement and definitive treatment of high voltage electric injury, and to remove focal areas of burn wound infection.

Advantages of burn wound excision include a reduction of the risk of invasive burn wound infection and certainly a reduction in the magnitude and duration of the burn size-related physiologic stress.

These indications and advantages must be balanced by a consideration of the blood loss, which has been estimated to be as high as 9% of the blood volume for each 1% of the body surface excised by the tangential technique (5), the risk of hepatitis and AIDS following the administration of blood and blood products, the uncertainty of graft "take" on subcutaneous fat, and the risk of sacrifice of viable skin in areas of partial-thickness burn, thus compounding the donor-recipient site disparity in the patient with extensive burn injury.

In summary, we identified a marked difference between surviving and nonsurviving patients with massive burn injury in terms of age, total burn size, extent of full-thickness burn, the presence of inhalation injury, and the ability of the patient to tolerate excision. The apparent advantage of excision, particularly in large burns, may be a reflection of patient selection, that is, the patient who is in condition to even be taken to the operating room.

In conclusion, we found that the major determinants of age, burn size, inhalation injury, and infection were unchanged in this group of patients, the ability to excise the burn wound was determined by the patient's response to injury, the predominant site of infection changed from the wound in prior years to the lung with the current techniques of wound care, bacterial burn wound infection has been replaced by nonbacterial infection with effective topical control and excision, and finally, when one comparies reported outcomes, comparable populations must be used for such an analysis, with

particular attention paid to inhalation injury and pneumonia across the populations being compared.

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SUMMARY OF CHAPTER III - WOUND CARE

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WE HAVE here a group of interesting papers that each bring up an interesting point. None could be classified as "rocket science," but then most burn work is not. The papers are all the product of some thought process and in some cases reflect a clinical bias that is often hard to filter out.

Dr. McManus tells us that no one really has any improved mortality figures with burn wound excision. I do not think anyone would take great umbrage with this statement, nor with the statements that the site of infection has changed from the burn wound to the lungs. The one totally intangible thing that cannot be evaluated in a paper such as this is the quality of the surviving patient. As we become more clever with conservative wound management, manipulation of topical agents, systemic antibiotics, and nutrition, we get more and more deep partial-thickness injuries to eventually heal. These injuries are the genesis of our major contractions and the source of all of our hypertrophic scars. I would agree that early excision may not save a lot of lives, but I would suggest that it prevents a lot of misery. This, of course, has to be weighed against the disadvantages of large amounts of blood loss and the telling effect of surgical stress. I could not disagree with anything that Dr. McManus said, but I think in evaluating the worthiness of early excision, the quality of survival must be factored in and this is very difficult to do.

Dr. Levine presents some of his early work in bilaminate skin substitutes. Dr. Levine should be marked as an early pioneer in this field. The observations he has made have been borne out with products such as Biobrane^{R}, and the later attempts at a collagen matrix for the dermis. The main point in Dr Levine's paper is that anything that is going to adhere to a fresh wound has to be a bilaminate structure. It must have an undersurface that the fibroblast and its friends can attach to and an outer impervious surface. I can well remember the dense adherence of Dr. Levine's skin substitute on planar surfaces. The major problem with all skin substitutes available so far is lack of conformability. When we achieve that in a collagen matrix that will eventually become a viable dermis, we will have gone a long ways towards solving the burn

wound management problem. A point not mentioned in the first two papers is that essentially any fool can cut off a hugh burn wound. It takes a great deal more talent to get that wound covered. Bilaminate skin substitutes that actually work will go a long ways towards helping this problem.

Dr. Chu demonstrated that there is another aspect to low voltage current and the mysteries of wound healing. This paper can join the legions of papers on the effect of low voltage on the nonunion of bones or the earlier healing of osseous lesions. Those applications of the modality have their strong supporters. It would appear to me that the silver-impregnated cloth with a current passing through it is yet another way to deliver the silver ion to the wound. It would seem to me that silver sulfadiazine is a better way, or at least an easier way, to deliver the silver ion. However, this is an interesting application. I think Dr. Chu should be congratulated for again documenting that early cooling of the burn wound makes the burn be less deep. This is something that we have wrestled with for a long time but now has been unequivocally demonstrated by other investigators. The more puzzling aspect of the study is the preservation of the added amount of dermis in the wounds that were not cooled. This would suggest that low voltage direct current in some way preserves a portion of the zone-of-stasis. Perhaps some day we can anticipate treating burns in silver space suits. Until that time, I would concentrate on the demonstrated advantage of early burn wound cooling.

Dr. Heimbach shows that there is a machine now that is better than an experienced physician in predicting what wound will heal in less than 3 wk. What I would applaude the most is his acceptance of the need to separate the burn wound into those that heal in 3 wk or less and those that take longer. This is a premise that is not always widely held and is very important in turning out acceptable postburn patients. The one thing that disturbs me is that the first time I saw Dr. Heimbach's presentation, the machine was suitcase size. Now it appears to be room size and I guess the next one will have to be pulled in an 18-wheeler. We are currently in the process of evaluating the laser doppler as an assessment of dermal circulation, and hopefully this will be a guide to what wounds will heal in 3 wk. In the meantime, we will await production of a practical imaging burn depth indicator that is really a good predictor. I would be delighted if I did not have to pick the wounds to be excised and not to be excised and could allow someone with no specialized training to do it. I would be even happier if I could have someone with no specialized training to do the excisions that are such an important part in turning out a good patient.

Lastly, Dr. Silverstein outlines the shortcomings of all enzymes that have been evaluated to date and has given us his concept of the ideal enzyme. He has also described how he uses

enzymes in the treatment of burns. I think that someone experienced in the use of the sutilains can use them as a method of debridement. My only long-time complaint has been too many people use them in a careless fashion, such as over too large parts of the body or for too long a period of time. Recently, we had the misfortune of having a 77-year-old female that had had sutilains applied to her hands and face for 14 days. One can say that they do not attack normal tissue, but in this case, her face was down to the bone on both structures and her burns were not that deep to start with. There has always been a great discrepancy between the detailing of the sutilains by their manufacturer and the proper clinical use of them. We use sutilains for chemical escharotomies on hands and in other very selected places. I would agree with Dr. Silverstein that we do not have the ideal enzyme yet, and when we do, we will be able to call it "Deadase" something that will remove necrotic tissue but not injure viable tissue. The big holdup at this time is the lack of a lipase that will digest dead fat.

I would like to thank all of the contributors. The papers were interesting and I enjoyed the opportunity to monitor the session.

CHAPTER IV - MISCELLANEOUS

ANESTHESIA MANAGEMENT OF BURN PATIENTS

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SURGERY MAY be required during the resuscitation, convalescence, or reconstructive phase of the burn patient's course. Patients who appear to be convalescing may revert to the resuscitation phase should another problem such as sepsis intervene. Each patient can present with unique problems involving monitoring, fluid administration, airway maintenance, and temperature control.

It may be very difficult to obtain a blood pressure using the standard cuff techniques, thus necessitating the use of noninvasive monitors or intraarterial lines. Almost any artery can be cannulated transiently to monitor blood pressure.

Venous access in major thermal injury can present a real challenge to the anesthesiologist. Almost any vein has been utilized, and indeed, areas of eschar may have to be excised to allow the insertion of central venous catheters in order to administer fluids to patients during operative procedures.

It is particularly important to remember that patients with major thermal injury are very susceptible to heat loss and therefore to cold stress. Efforts should be made in the operating room to conserve heat in the patient. Fluid should be warmed, heated humidity added to the anesthesia circuit, and the room temperature kept warm.

Difficulties with the patient's airway will depend upon the patient's site of injury and period in the postinjury course. Should operative intervention be necessary during the acute resuscitation phase, the patient may present with massive edema of the upper airway and face, and because of the relative instability of the patient at this time, endotracheal intubation is nearly always indicated for any surgical procedure requiring anesthesia. During the postresuscitation phase, however, patients are frequently unable to open their mouth wide enough for intubation to be accomplished, a situation which may be necessary if surgery around the face and neck is anticipated. Therefore, awake, blind, nasal intubation, the use of the flexible laryngoscope, or a curved optical stylet may be necessary in order to facilitate intubation prior to surgery. Repair of burn contractures, such as neck flexion contractures, and microstomia during plastic and reconstructive surgery may also present with problems in airway management. Occasionally, it is necesasry to administer ketamine to release contractures just prior to intubation. This can be a very anxiety-provoking procedure. There is also

the problem of protecting suture lines and skin grafts during the emergence of the patient from anesthesia.

The types of procedures performed during early burn therapy are usually for treatment of concurrent injuries, exploration of the burn wound to determine the extent of injury following electrical injury, and burn wound excisions. Following resuscitation, the most common procedures consist of multiple debridements, further excision of the burn wound, and skin grafting. At any point during this time, the patient may develop other medical problems, such as upper gastrointestinal bleeding, suppurative thrombophlebitis, and cholecystitis, which may require operative intervention.

During the reconstructive phase, a patient may require multiple procedures which involve skin grafts, flaps, and free pedicle grafts (Table I). Therefore, anesthesia management needs to be tailored to the patient, and patients with thermal injury will present with some additional considerations. On the average, they will require several anesthetics, and although there is thought to be a decreased sensitivity to halothane hepatitis in these patients, other agents are probably more suitable for multiple anesthetic administration (Tables II and III). In addition, succinylcholine may lead to severe hyperkalemia, and therefore, pancuronium bromide is frequently used as a rapid-acting, nondepolarizing agent for intubation of thermal injury patients (atracurium besylate, 0.4 mg/kg, vecuronium bromide, 0.1 mg/kg).

Recently, it has been demonstrated that patients with thermal injury have alterations in their response to drugs used in administering anesthesia. A reduced sensitivity to diazepam and thiopental have been observed (2). An increased dosage necessary to produce a 95% twitch depression has been shown for d-tubocurarine, metocurine iodide, and pancuronium bromide. With the latter drug, an increase in dose also resulted in prolonged action, perhaps related to the reduced liver metabolism in thermal injury.

Often the various regional techniques cannot be used in major thermal injury because of either the location of the burn or the extent of the anticipated surgery. Ketamine can be very useful in burn anesthesia. The advantage of ketamine is the patient will usually ventilate adequately while achieving a surgical plane of anesthesia. Ketamine, however, does not protect against regurgitation and aspiration, and patients who are going to be given a ketamine anesthesia should be kept NPO prior to surgery. Ketamine will also result in an increase in the oral secretions. A drying agent should be administered if multiple doses of ketamine are anticipated. The induction dose is approximately 2-5 mg/kg IV and maintenance doses are one-quarter to one-half that dose. An intramuscular dose of 1-2 mg/kg has been useful for minor debridements and procedures

TABLE I. Recent Trends in Operative Procedures*

Procedure	1980		1981		1982		1983		1984	
	Number	%	Number	%	Number	%	Number	%	Number	%
Excision	269	37.36	212	36.38	257	33.29	196	42.00	323	41.10
Autograft	318	44.17	293	50.69	405	52.46	203	43.60	371	47.20
Orthopedic	38	5.28	23	3.98	31	4.01	22	4.70	30	3.80
Chrondrectomy	4	0.56	3	0.52	0	0.00	2	0.40	4	0.50
Eye and Lid	17	2.36	3	0.52	14	1.81	8	1.70	18	2.30
Intraabdominal	1	0.14	1	0.17	6	0.78	2	0.40	5	0.60
Plastic	5	0.69	3	0.52	15	1.94	2	0.40	5	0.60
Other	68	9.44	40	6.92	44	5.70	31	6.70	31	3.90
TOTAL	720	100.00	578	100.00	772	100.00	466	100.00	787	100.00

*From Jirka AJ, Schmidt SI, McManus WF, et al (3). Table used with permission from the authors.

TABLE II. Pattern of Anesthesia Administration*

Agent	1981		1982		1983		1984	
	Number	%	Number	%	Number	%	Number	%
Enflurane	252	62.38	335	62.97	184	63.23	290	62.91
Ketamine	104	25.74	169	31.77	66	22.68	88	19.09
Halothane	16	3.96	1	0.19	0	0.00	0	0.00
Nitrous oxide	20	4.95	8	1.50	22	7.56	27	5.86
Local	10	2.48	15	2.81	13	4.47	14	3.04
Other	2	0.49	4	0.75	6	2.00	42	9.11
TOTAL	404	100.00	532	100.00	291	100.00	461	100.00

*From Jirka AJ, Schmidt SI, McManus WF, et al (3). Table used with permission from the authors.

TABLE III. Overall Anesthetic Patient Data (1979-84)*

Year	Number of Patients	Number of Patients Anesthetized	Percentage of All Patients	Total Anesthestics Given	Average Anesthetics Per Patients Anesthetized
1971	301	179	59.50	475	2.65
1972	301	183	60.80	575	3.14
1973	273	141	51.60	377	2.67
1974	226	123	54.40	380	3.09
1975	254	142	55.90	490	3.45
1976	277	139	50.20	476	3.43
1977	242	129	53.30	344	2.67
1978	268	151	56.30	435	2.88
1979	267	161	60.30	554	3.44
1980	243	148	60.91	531	3.59
1981	208	127	61.06	404	3.18
1982	231	151	65.37	532	3.52
1983	179	98	54.75	291	2.97
1984	190	139	73.16	461	3.32

*From Jirka AJ, Schmidt SI, McManus WF, et al (3). Table used with permission from the authors.

which will only last approximately 20 min. This will provide intense analgesia and profound amnesia.

Anesthesia in patients who have sustained a major thermal injury presents an unique challenge from the initial resuscitation to the completion of reconstructive surgery. Adequate understanding of these problems will result in better anesthetic care.

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THE RISE AND FALL OF *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa became a commonly reported laboratory finding after the introduction of the first generations of modern antimicrobial agents. With today's knowledge of the natural and acquired mechanisms of resistance to agents such as penicillin and sulfonamides, it seems obvious that Pseudomonas was the natural fit for the niche occupied by Group A beta hemolytic streptococci and other susceptible burn pathogens with limited mechanisms of resistance. This microbial and clinical evolution was recognized and first reported in the 1950s from this Institute by Drs. Reiss, Artz, Riveria, Barnes, Tumbusch, Graber, and others. Investigators from this Institute, such as Drs. Moncrief, Teplitz, Mason, Lindberg, Switzer, Curreri, Pruitt, and others past and present also played a major role in documenting effective therapy for Pseudomonas infections. The first statistically demonstrable antipseudomonal advance in burn treatment, topical antimicrobial therapy with mafenide acetate, is our reward for their efforts.

This paper will present this Institute's most recent experience with *Pseudomonas aeruginosa*. During the past 10 years, Pseudomonas burn wound invasions and blood isolations have markedly decreased. Figures 1 and 2 show that these two clinical events have essentially disappeared. I use the word disappeared rather than eliminated because we do not know the specific reason for the decline. No obvious advance in chemotherapy or other therapy can be associated. The severity of injury of the patient population as measured using Dr. Mason's logistic regression formulae (1) has not changed to a degree that would suggest a decrease in susceptibility. The mean severity indices for patients admitted since 1977 are presented in Figure 3.

Several facts that do relate temporally to the changes in Pseudomonas wound and blood infections are an increased emphasis on infection control, the use of a computerized epidemiological data base, and the change in physical isolation that occurred when the Institute's open ward intensive care unit, Ward 14A, was rebuilt to contain primarily single-bed isolation rooms.

The floor plan of the new Ward 14A intensive care unit is presented in Figure 4. The bed changes can be best realized by comparing the floor plan of the Ward 14A to Ward 14B. Before the renovation, the two wards were very similar, with the old Ward 14A intensive care unit being an open 8-bed ward as shown

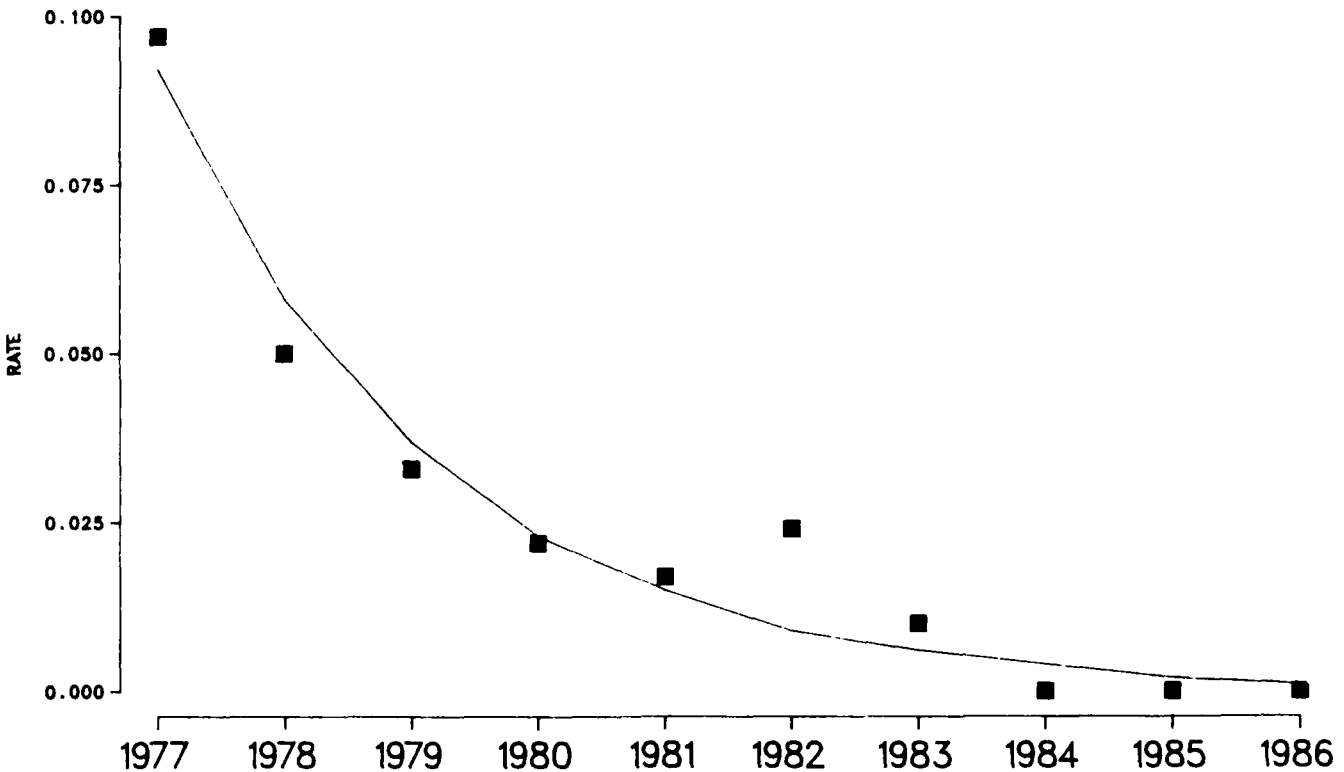

FIGURE 1. Curvilinear regression of the rate (cases/100 admissions) of Pseudomonas aerguinosa burn wound sepsis by year.

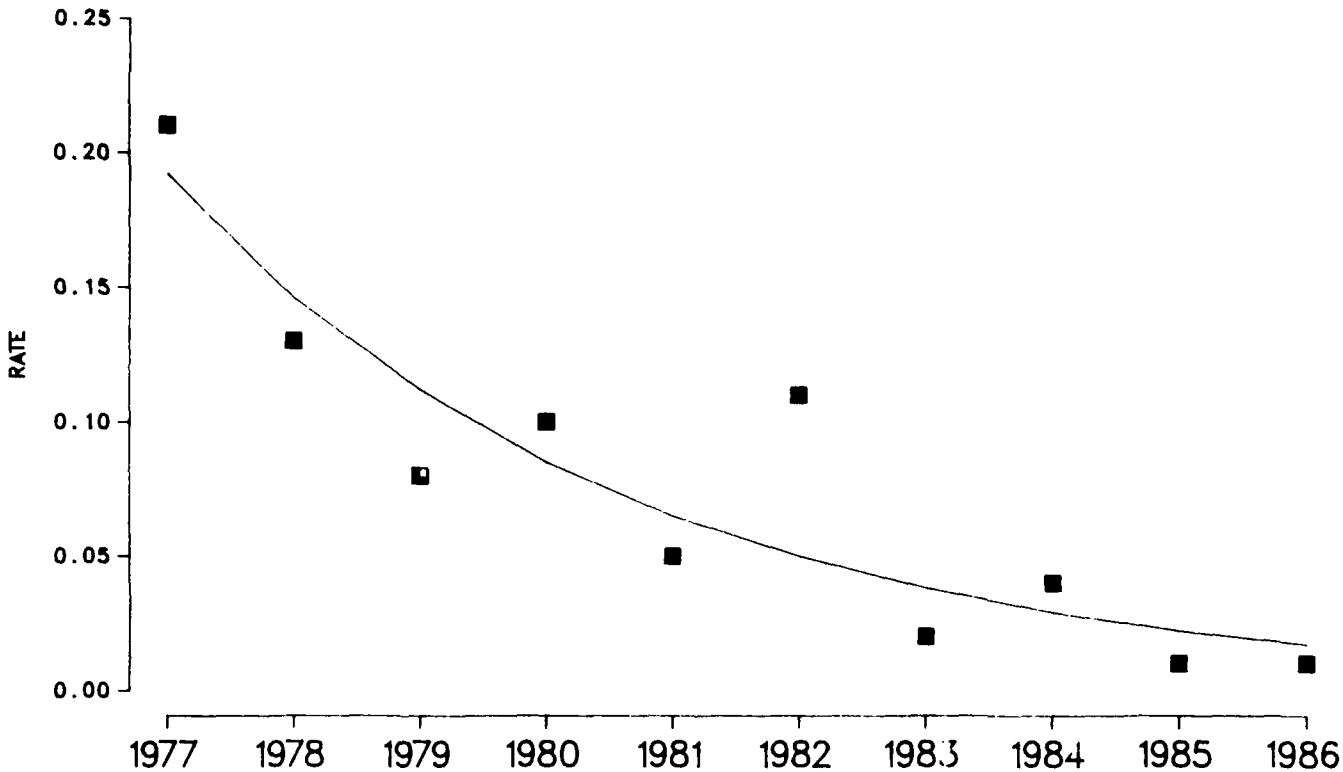

FIGURE 2. Curvilinear regression of the rate (cases/100 admissions) of Pseudomonas aerguinosa bacteria by year.

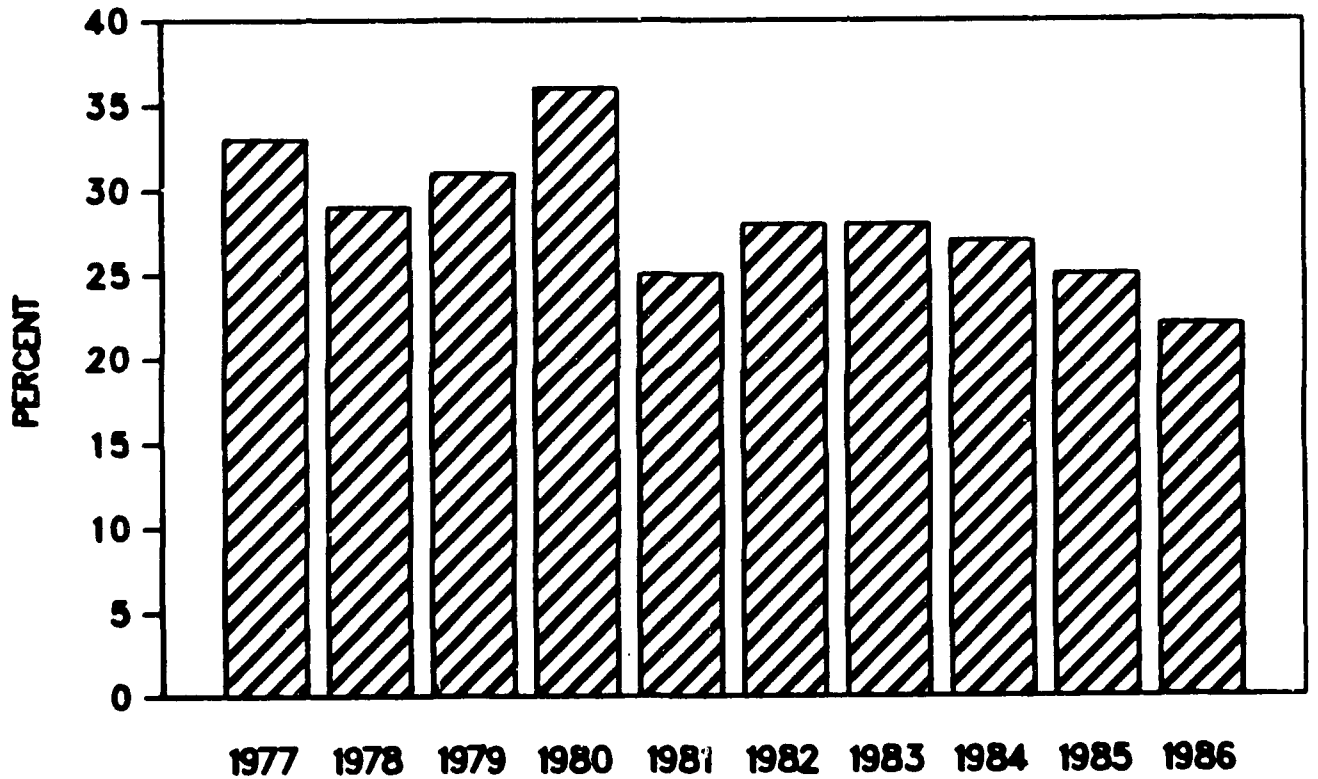

FIGURE 3. Distribution of mean severity of injury (probability of mortality) as a percentage from 1977-86.

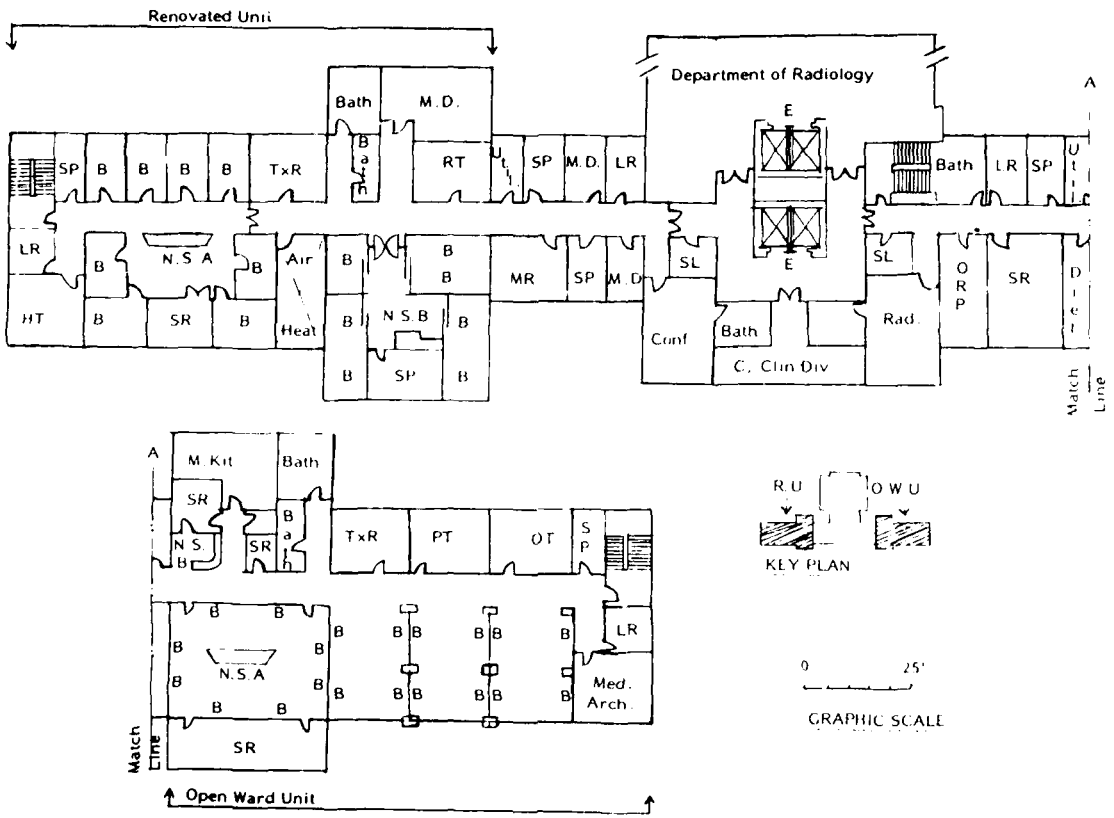

FIGURE 4. Floor diagram of individualized intensive care unit rooms on new Ward 14A (renovated unit) and open-ward intensive care unit Ward 14B (open-ward unit).

for Ward 14B. During construction, Ward 14A was closed and Ward 14B was used as the intensive care unit with 8 open beds. The remainder of Ward 14B was used as a less intensive care area and a convalescence ward. Ward 15A was used as a spill over convalescence ward.

Prior to the opening of the new Ward 14A intensive care unit, plans were established to attempt elimination of two endemic bacterial strains and to compare microbial flora colonization of patients admitted to the new intensive care unit and the old intensive care unit. Microbial surveillance had documented the extended presence of a Pseudomonas aeruginosa strain that was both an unusual serotype (international 15) and had a consistent resistance pattern to the aminoglycosides and most antipseudomonal beta lactam antibiotics. This strain had been present in the intensive care area for more than 5 yr. In addition to the identifiable Pseudomonas, a Providencia stuartii strain with a characterized antibiotic pattern and plasmid profile was also present in the intensive care area for more than 2 yr. Both of these organisms were present on the Ward 14B intensive care unit during the reconstruction period. Part of the plan was to only allow new admission to the new ward. The flow diagram for new patients is shown in Figure 5. When the ward was ready, fresh patients were admitted directly to the new ward without contact with patients on Ward 14B. In addition, a burn care nursing team was assigned with the new patient to the new ward. These teams were formed when the clinical personnel were returning to duty after 1 or more days of nonpatient care time. Clinical care personnel that were required to work on both wards during the same day organized their work so that the work on the new intensive care unit was completed first and the personnel would not return that day after working with the Ward 14B patients.

Microbial surveillance techniques which had been in continuous use for several years prior to the construction were continued. These techniques included the plating of specimens in addition to standard isolation media onto a MacConkey's agar plate containing 20 μg/ml gentamicin sulphate. The gentamicin plate was not inhibitory for the two targeted endemic strains. The first 25-patient cohort admitted to the new unit was compared to the cohort of the last 25 patients admitted to the old open-bed Ward 14B. The time courses of admission of patients into the 2 cohorts are presented in Figure 6. The dispersal of the 2 cohorts to convalescence or death is shown in Figure 7. The details of the microbiologic changes measured during this study are presented elsewhere (2-4).

As a summary, the two test organisms were not found in the new cohort and the organisms and the Providencia plasmid were lost from the entire Institute with the eventual disposal of the original patient population. In addition to the loss of the two known endemic organisms, overall reduction of resistance of Gram-negative flora has continued. A comparison

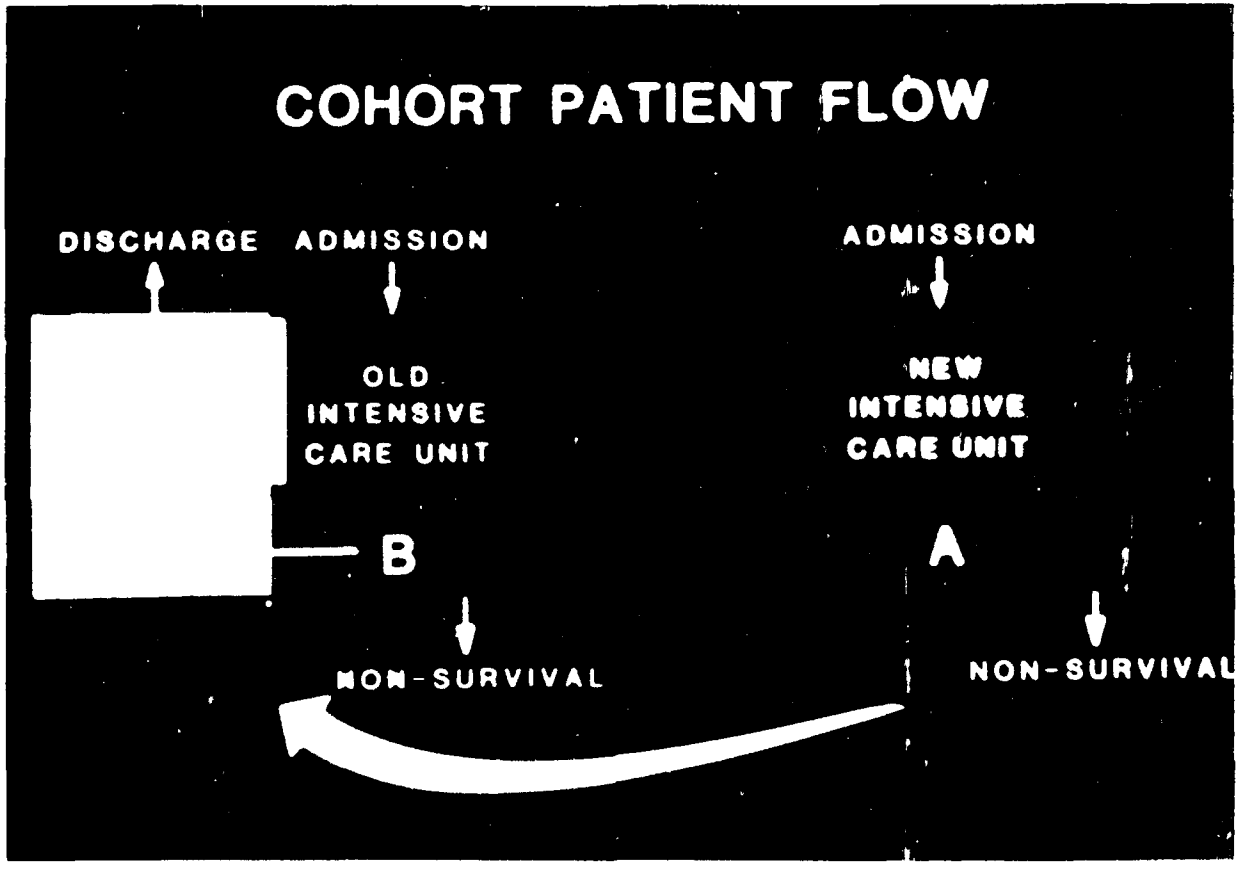

FIGURE 5. Admission scheme for new intensive care unit. New admissions were not mixed with open intensive care unit patients until discharge from intensive care.

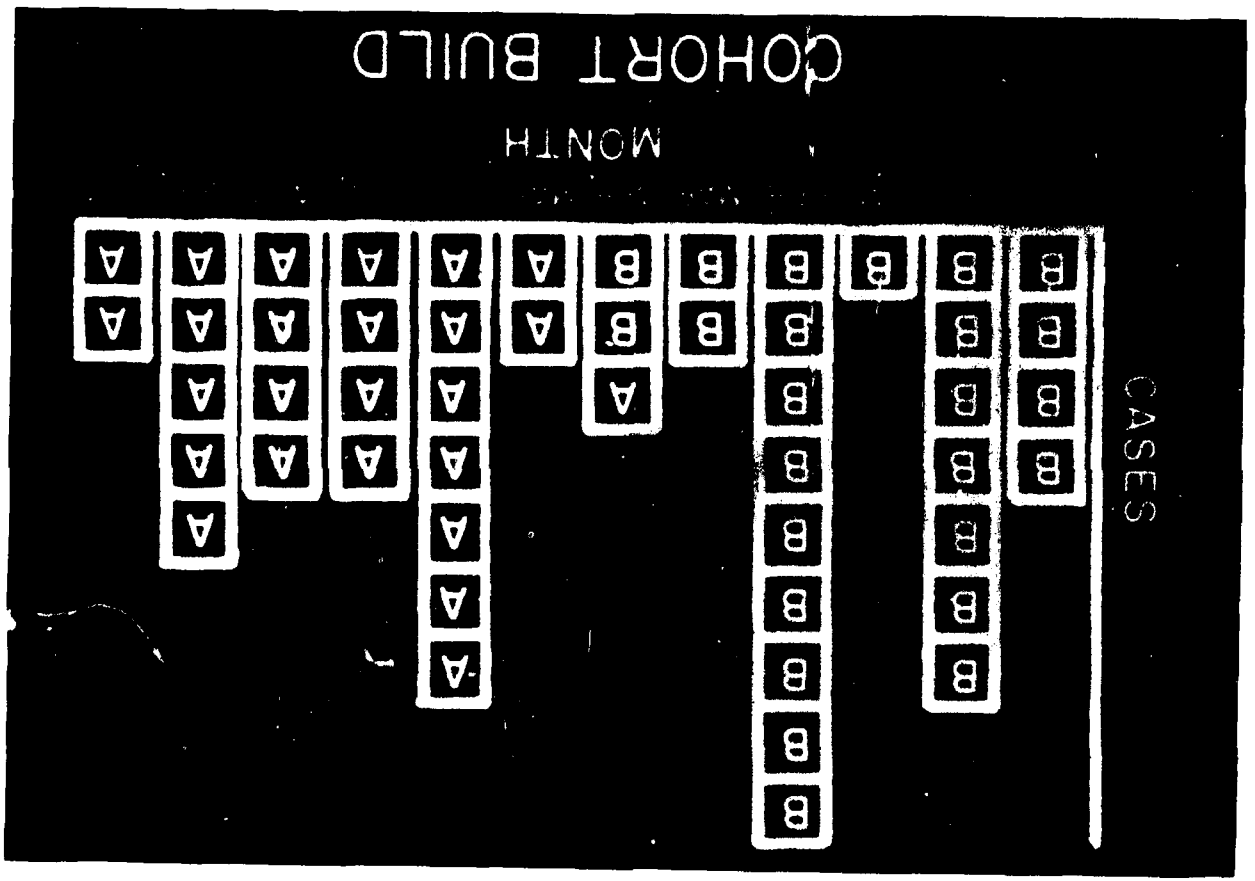

FIGURE 6. Case (block) diagram of admission months of new ward cohort (A) and old ward cohort (B).

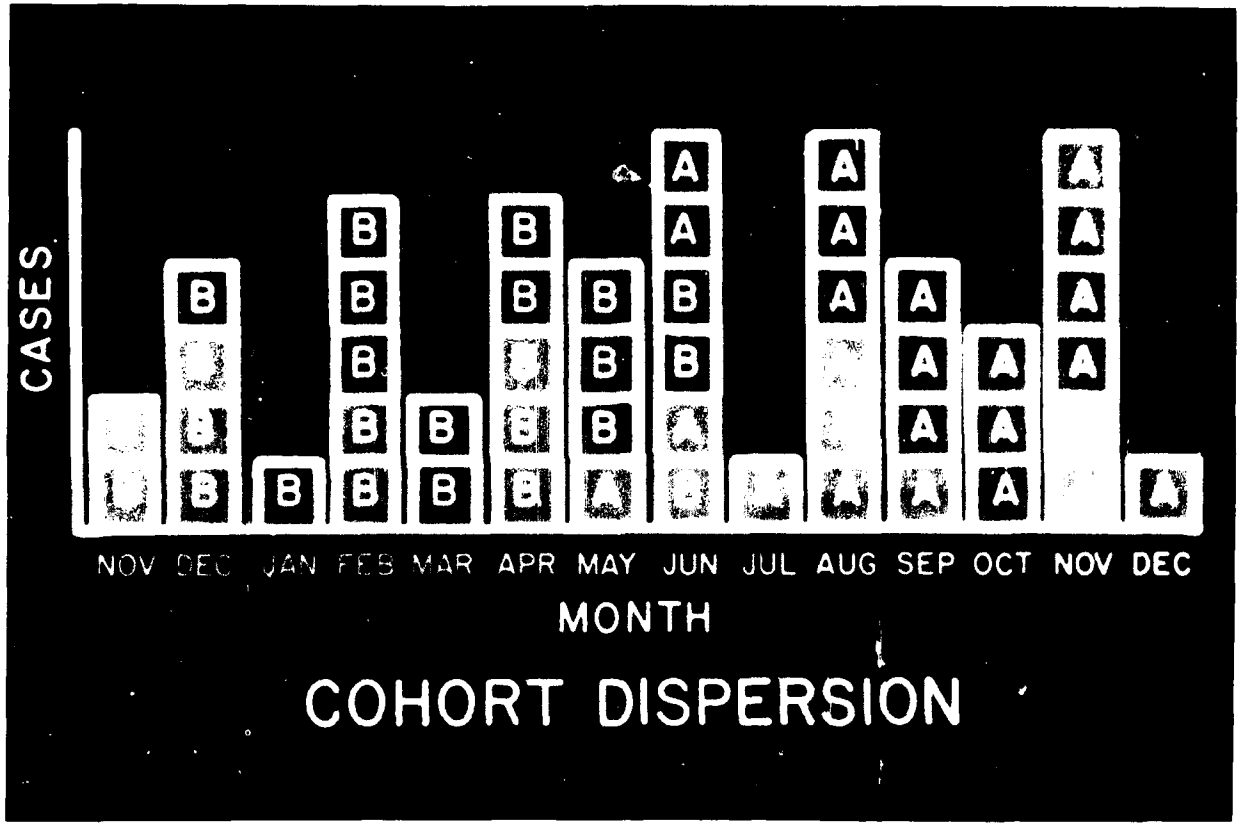

FIGURE 7. Case (block) diagram of disposition month from new ward cohort (A) and old ward cohort (B).

of the Gram-negative rod antibiotic sensitivities for the year prior to the new ward and this year to date is presented in Table I. Figures 8 and 9 show the yearly changes noted for Pseudomonas aeruginosa to gentamicin and sulfonamides, two markers used as possible indicators of hospital-acquired multiply resistance strains and plasmids. The changes in flora noted after the availability of single-room isolation have also been reflected in types of organisms causing infections. Figure 10 depicts organisms causing infections during the last year of open bed intensive care. As can be seen, Pseudomonas aeruginosa was the most common cause of infections. Candida rugosa, an uncommon Candida species, was the second most common pathogen. Figure 11 shows the Institute's most recent infection data. Pseudomonas infection has dropped to <5%. The most common Pseudomonas aeruginosa infection is no longer wound or blood invasion; pneumonia is the most common site but the incidence is <10% of all pneumonias.

TABLE I. Antibiotic Activity Among Gram-Negative Isolates

	1982 (% Resistant)	1987 (% Resistant)	P Value
Amikacin	25	6	< 0.0001
Gentamicin	49	8	< 0.0001
Tobramycin	66	16	< 0.0001
Ticarcillin	45	39	< 0.01
Mezlocillin	29	20	< 0.01
Piperacillin	18	17	NS
Sulfadiazine	76	44	< 0.0001

As a final comment on Pseudomonas aeruginosa at this Institute, the incidence of infection has obviously dropped, but the organism is still present. As shown in Figure 12, the percent of patients colonized decreased slightly during the first year of the new intensive care unit but has remained above 20% since that time. This is an important observation. Pseudomonas aeruginosa is still present but is no longer causing the frequency of infections previously noted. I believe this is the direct result of the loss of Pseudomonas aeruginosa's primary advantage, strain persistence. We have eliminated or greatly reduced the frequency of cross-contamination from patient to patient of strains that have acquired mechanism to survive in the burn care environment. The breaking of transmission not only prevents the accumulation of virulence mechanism but also increases the frequency of nonhospital strains that, when treated, are more easily controlled by available methods.

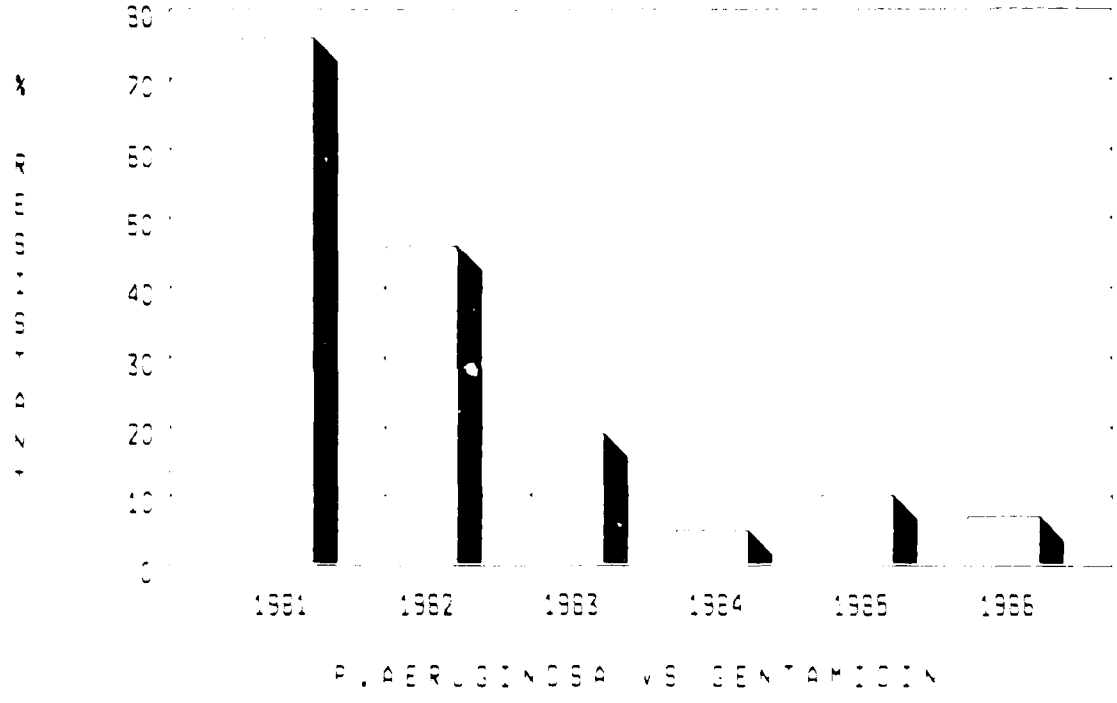

FIGURE 8. Percent of Pseudomonas aeruginosa strains sensitive to gentamicin as function of year of isolation.

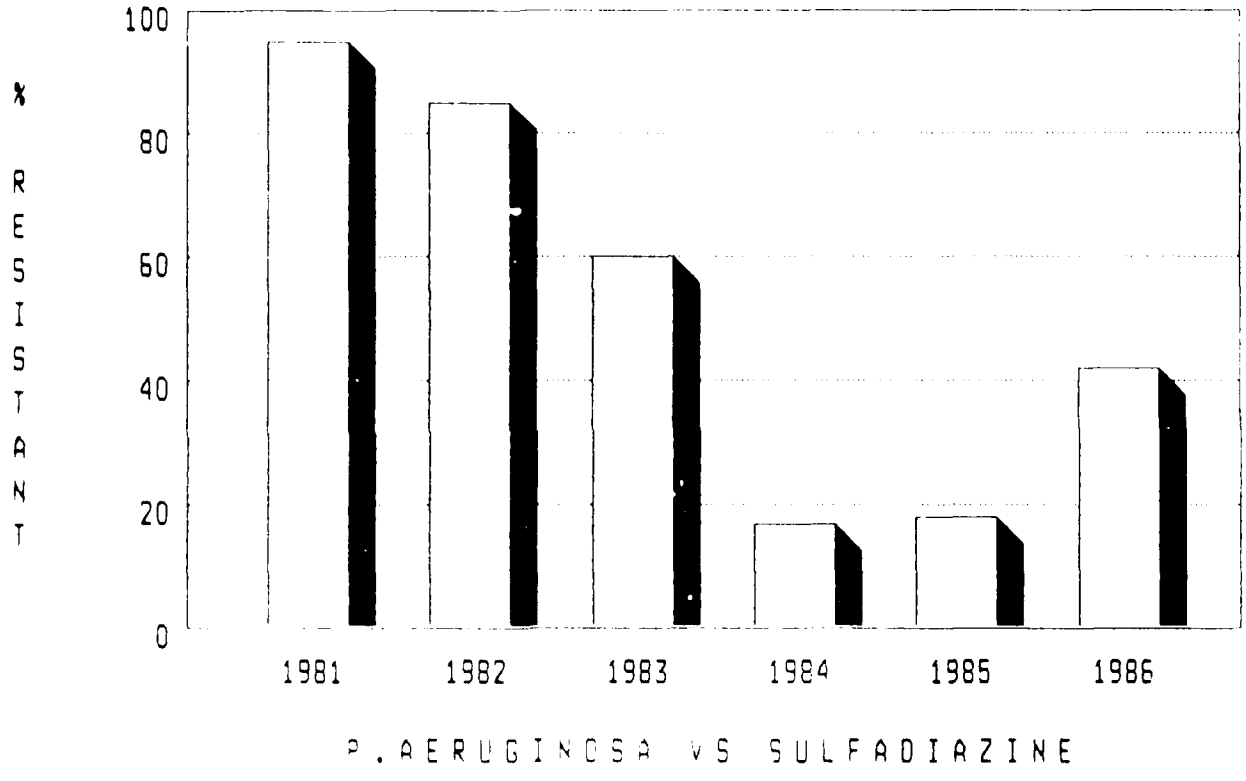

FIGURE 9. Percent of Pseudomonas aeruginosa strains sensitive to sulfonamides as a function of year of isolation.

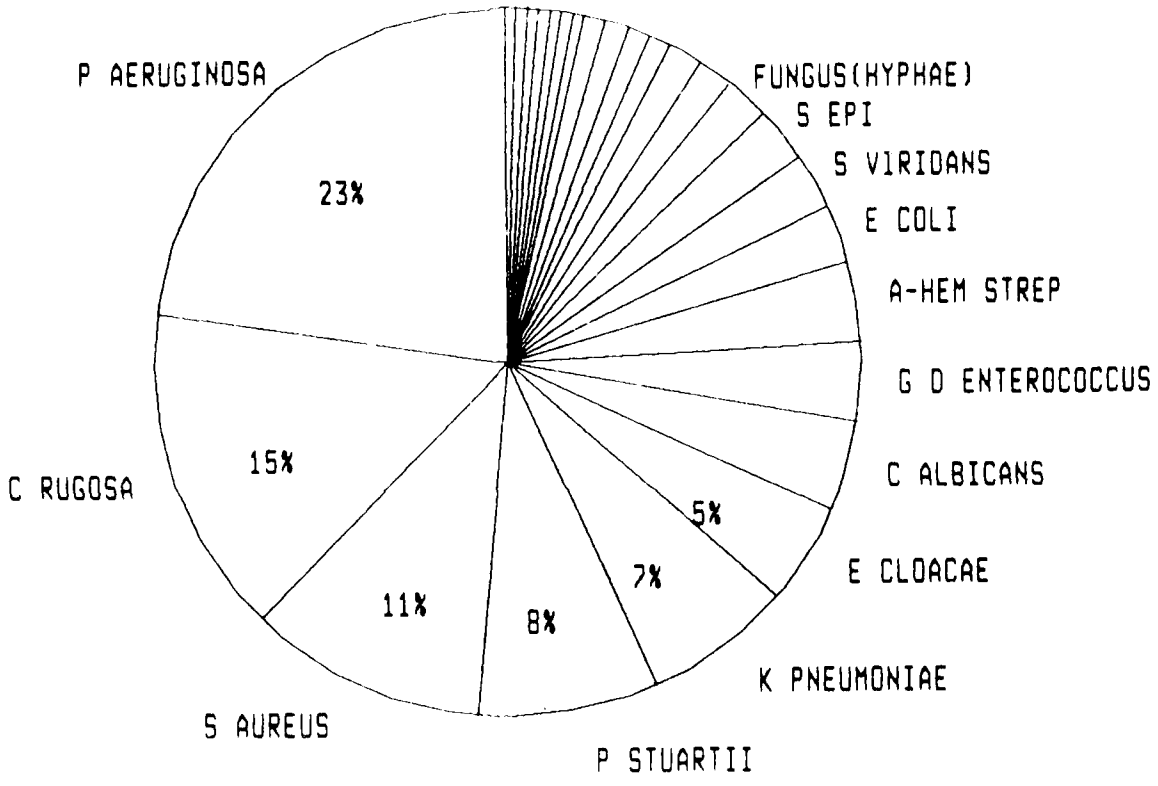

FIGURE 10. Organisms causing infections during the 12-month period prior to the opening of the new Ward 14A.

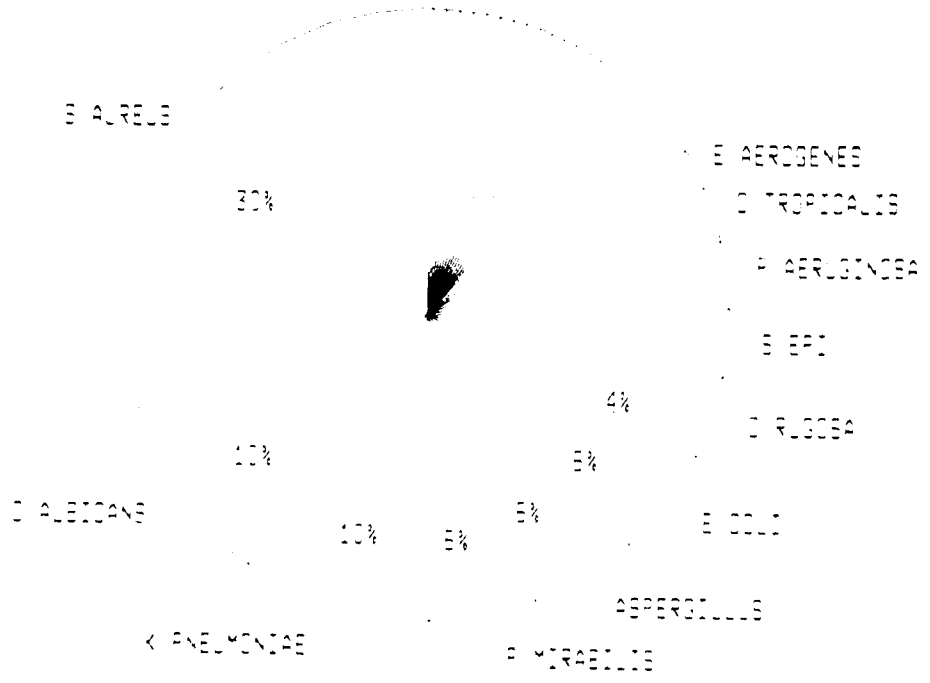

FIGURE 11. Organisms causing recent infections on Ward 14A.

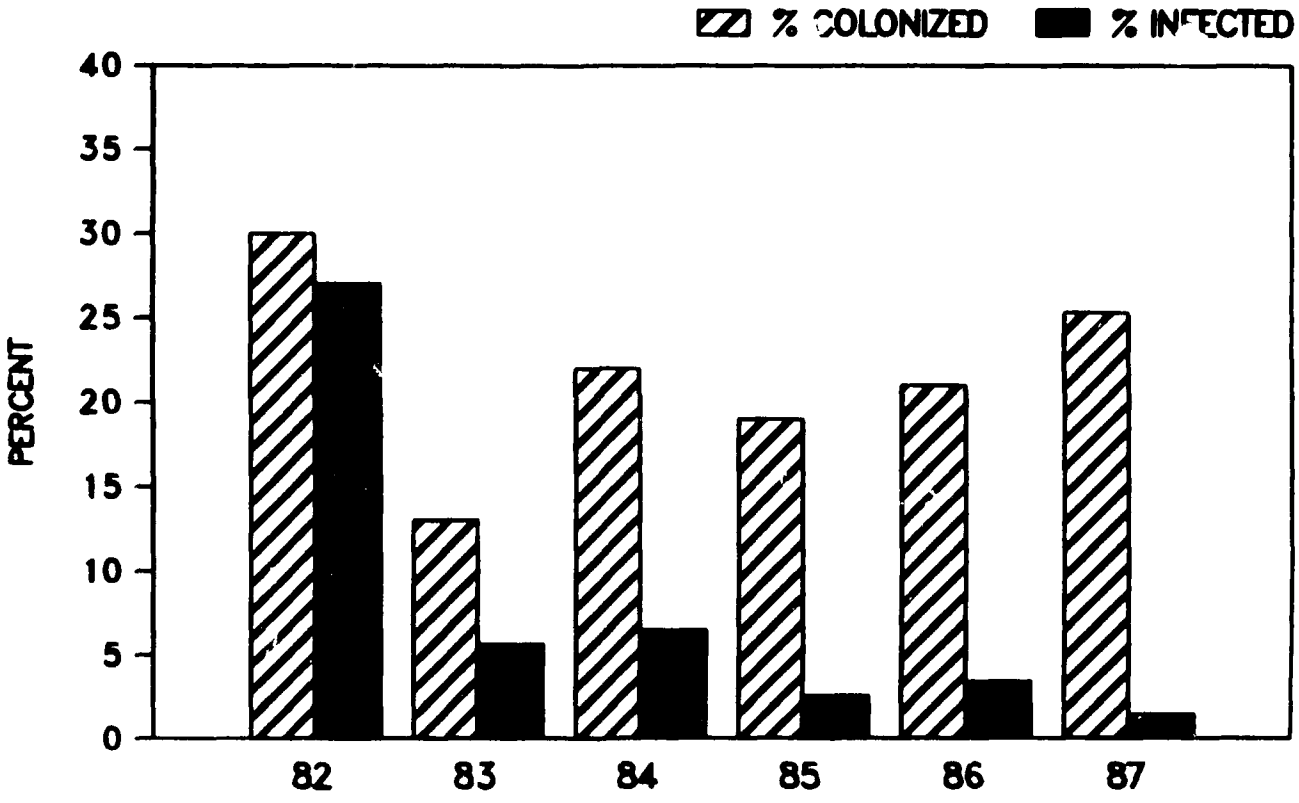

FIGURE 12. Frequency of colonization and infection with Pseudomonas aeruginosa by year from 1982-87.

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HISTOLOGIC CLASSIFICATION OF BURN WOUND INFECTIONS

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BURN WOUND infection is a major problem for severely burned patients in most burn centers. Even with modern topical and systemic chemotherapeutic agents and sophisticated surgical management, wound infection is frequently the cause of patient demise.

Quantitative microbiological culture of burn wound biopsies has been used in many burn institutions for several decades (1); however, comparison with histopathological examination of biopsies has shown the latter to be more clinically relevant and a superior evaluation of burn wound infection (2). Early diagnosis of wound infections using the frozen section technique followed by rapid section technique have been well documented (3-4).

Classification systems of burn wound infections have been proposed by several authors (5-7). Tremendous complexity and lack of correlation with the clinical situation have discouraged the use of these classification systems.

A new system of classification using staging of the burn wound was initiated by our Institute in the mid-1980s. This system can be applied to autopsy and biopsy specimens. This classification system is divided into 2 stages. In Stage I (burn wound colonization), the organism is limited to the nonviable eschar. In Stage II (invasive burn wound infection), the organism is present in the viable tissue under the eschar. Each stage is subdivided into 3 subgroups. Burn wound colonization is subdivided into Stage Ia (superficial colonization), Stage Ib (deep colonization), and Stage Ic (proliferation of the organism at the interface between nonviable and viable tissue). Invasive burn wound infection is subdivided into Stage IIa (microinvasion), Stage IIb (deep extensive invasion), and Stage IIc (vascular and perineural invasion).

We have applied this classification system to all burn wound biopsy and autopsy cases examined at our Institute over the last 8 yr (1978-86). A total of 252 autopsies were evaluated and 25 cases of metastatic mycotic abscesses were identified. The frequency of mycotic abscess formation at each biopsy stage was 1 in 19 (5.3%) for Stage Ia, 1 in 25 (4.0%) for Stage Ib, 3 in 18 (16.7%) for Stage Ic, 8 in 34 (23.5%) for Stage IIa, 5 in 10 (50.0%) for Stage IIb, and 7 in 7 (100%) for Stage IIc. The staging system was alsc applied to wound biopsies without limitation to specific organisms. The staging

of burn wound biopsies from 166 patients was compared to patient outcome. The mortality rate in each stage was 6 in 19 (31.6%) for Stage Ia, 9 in 24 (37.5%) for Stage Ib, 10 in 17 (58.8%) for Stage Ic, 24 in 31 (77.4%) for Stage IIa, 8 in 9 (88.9%) for Stage IIb, and 5 in 8 (62.5%) for Stage IIc.

The depth of the infection in the new 2-staged classification system of burn wounds was strongly correlated to internal mycotic abscess formation and death of the patients evaluated. Surgical excision of the invaded tissue and systemic therapy is indicated in Stage II infections to prevent progression of the disease process. This staging system is currently the best choice for monitoring burn wound infection and preventing metastatic internal organ abscesses or burn wound-related septicemias.

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PREVENTION AND TREATMENT OF HYPERTROPHIC SCARS

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PHYSICIANS caring for burned patients should be aware of the long-term results of their therapy regarding future quality of life for the patient. Certainly, survival must be the primary goal, but this does not preclude care taken to decrease scar formation and the resulting deformities.

Formation of Hypertrophic Scars. The processes of healing in burn wounds are conducive to the formation of hypertrophic scars, as they are characterized by a marked increase in vascularity, fibroblasts, myofibroblasts, collagen deposition, interstitial material, and edema (1). When the red coloration of the healed burn wound lessens over the first 2 months, hypertrophic scar formation is unusual. However, the one that remains highly vascular at the end of 2 months and becomes progressively firmer will be hypertrophic by the third or fourth month.

The fibroblast found in early healed burn wounds is characterized by having a rich array of dilated rough endoplasmic reticulum, indicating a high degree of synthetic activity. Biochemical studies revealed a significant increase in mucopolysaccharides. Chondroitin sulfate B, the one most commonly found in the dermis of normal skin, was replaced in hypertrophic scars by a relatively increased amount of chondroitin sulfate A, which is usually associated with firm tissues such as cartilage. This increased amount and changes in the mucopolysaccharides appear to increase the adhesive nature of the scar. The burn patient who is allowed to be in a flexed position will develop flexion contractures. Therefore, the cardinal rule in burn care is that the position of comfort is the position of contracture.

Hypertrophic scars contain many fibroblasts with contractile-like elements, 40-80 Å in diameter, in the cytoplasmic matrix as well as irregular surface features and deeply indented nuclei, indicating contraction of the cells. These cells are called myofibroblasts because they can resemble smooth muscle cells physiologically and biochemically. Their cross-reactivity with a human anti-smooth muscle serum antibody suggests an actinomysin component with contractile properties. These contractile-like cells are increased in the deeper portions of the hypertrophic scar in close proximity to observed capillary beds. As these cells contract, the resulting relief of stress upon the adjacent collagen fibrils allows these fibrils to form a wavy pattern and supercoils of collagen. The contraction in the depth of the dermis forces outward or "piling up" of the scar, resulting in the typical

elevated hypertrophic scar. The hypertrophic scar has an increased water content and adequate lymphatic function cannot be demonstrated; thus, these scars are examples of localized lymphedema. The edema can result in mast cell degranulation with release of histamine and prolongation of the inflammatory response.

These vigorous contractile properties of the hypertrophic scar described has resulted in a cardinal rule in burn care, i.e., the burn wound will shorten until it meets an opposing force.

Prevention of Hypertrophy and Contracture Formation. Scar contracture and hypertrophic scar formation can be markedly lessened by proper positioning of the patient, utilization of splints to maintain good position of all joints, vigorous exercise to sustain optimal range of motion, and long-term constant pressure dressings. The use of pressure >25 mmHg on the healed burn wound will decrease the vascularity, depress the tissue partial pressure of oxygen, decrease the amount of mucopolysaccharides, especially chondroitin sulfate A, and decrease the cellular response as well as the collagen deposition, the localized lymphedema, and degranulation of the mast cells. Pressure also improves the arrangement of collagen bundles with "spreading out" effect on the scar, resulting in decreased scar hypertrophy and contracture formation.

The primary goals of burn care are summarized in a letter from the mother of a burned child which says "Thank you for helping my child to live. He enjoys every minute of it."

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THE EFFECT OF HEMOGLOBIN CONCENTRATION ON CARDIAC OUTPUT IN HYPERMETABOLIC BURN PATIENTS

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WHAT ARE the hemodynamic implications of changes in hemoglobin concentration (Hb) in hypermetabolic patients? The answer to this question has obvious clinical relevance in view of the the following observations:

1. The metabolic rate can be twice normal or greater during the hypermetabolic phase in patients with large burns (1).

2. Extremely high levels of cardiac output (CO) (up to 20 L/min) have been reported in patients with large burns (2). It has also been postulated that a component of the increase in CO is due to "shunting" of blood at the level of the microcirculation, i.e., nonnutrient blood flow (NBF) (3).

3. Cardiopulmonary failure remains a major impediment to survival in many middle age and elderly burn patients.

In order to study the above question, the interrelationship of cardiac index (CI) and Hb was examined, both from a theoretical point of view using a mathematical model for NBF and from clinical observations. NBF is that portion of the CO that is excluded from oxygen exchange at the level of microcirculation. It can represent left to right intracardiac shunting, large vessel arteriovenous fistulae, true anatomic arteriovenous shunting in the microcirculation, altered perfusion of capillary beds, and nonnutrient microcirculatory flow from cellular failure to utilize oxygen. The following equation for NBF (where both oxygen delivery (DO_2) and oxygen consumption ($\dot{V}O_2$) are greater than that predicted in the basal state for the age and sex of the patient) was used (4):

$$\% \text{ NBF} = \{[(DO_2)_2/(DO_2)_1 - 1] - [(\dot{V}O_2)_2/\dot{V}O_2)_1 - 1]\} \times 100$$

Subscript 1 values are those in the basal state for age and sex of the patient. Subscript 2 values are those of the patient. The CI component of $(DO_2)_2$ is solved from the above equation:

$$\% \text{ NBF}/100 = S \text{ (fraction of CO that is "shunted")}$$

$$S = [(DO_2)_2/(DO_2)_1 - 1] - [(\dot{V}O_2)_2/(\dot{V}O_2)_1 - 1]$$

$$S = (DO_2)_2/(DO_2)_1 - (\dot{V}O_2)_2/(\dot{V}O_2)_1$$

Rearranging: $(DO_2)_2 = [S + (\dot{V}O_2)_2/(\dot{V}O_2)_1](DO_2)_1$

$$CI = \frac{[S + (\dot{V}O_2)_2/(\dot{V}O_2)_1](DO_2)_1}{[(Hb)(SaO_2)(1.36) + PaO_2\ (0.0031)] \times 10}$$

This mathematical model, which interrelates CI, Hb, NBF, and the metabolic rate (the ratio of $(\dot{V}O_2)_2/(\dot{V}O_2)_1$, and whose prediction of high CO fits with observed clinical findings, was used to study the interrelationship of CI and Hb with varying degrees of NBF in an 18-yr old male (basal DO_2 = 560 ml/min/m^2, basal $\dot{V}O_2$ = 140 ml/min/m^2) who has normal arterial oxygenation and a $\dot{V}O_2$ of twice normal (280 ml/min/m^2). The results are shown in Figure 1. An inverse relationship between CI and Hb is shown at three different levels of NBF. This inverse relationship between CI and Hb has been observed in a small group of unequivocally septic burn patients who had NBF in the range of 25-70% (4).

Further clinical studies in another subset of critically ill burn patients (19 hemodynamic-oxygenation profiles from 11 patients) are reported where $\dot{V}O_2$ was elevated and there was utilization of reserve oxygen transport capacity, instead of excess oxygen delivery relative to oxygen demand (5).

The range of VO_2 was 180-325 ml/min/m^2 (mean = 218.6 $\pm$ 41.9). The range of CI was 1.8-6.1 L/min/m^2 (mean = 3.55 $\pm$ 1.0). The range of Hb was 9.1-15.0 g % (mean = 12.0 $\pm$ 1.7 g %). The range of utilization of reserve oxygen transport capacity was 30-118% (mean = 65 $\pm$ 26%). The theoretical maximum for utilization of reserve oxygen transport capacity is 300%. The following equation was obtained by linear regression analysis:

$$CI = -0.47\ (Hb) + 9.19\ (n=19)$$

$$\text{Correlation Coefficient} = -0.78$$

There is a significant inverse relationship ($P < 0.001$) between CI and Hb in the small number of patients studied in this subset of critically ill burn patients. This inverse relationship was found not only in hypermetabolic burn patients who are utilizing reserve oxygen transport capacity, but in those patients where oxygen delivery is in excess relative to oxygen demand, i.e., where there is NBF. Both in the data generated by using a mathematical model for NBF and in the two clinical studies reported, the incremental increases in CI associated with a decrease in Hb are similar, but as NBF increases, a higher CI is required for a given Hb.

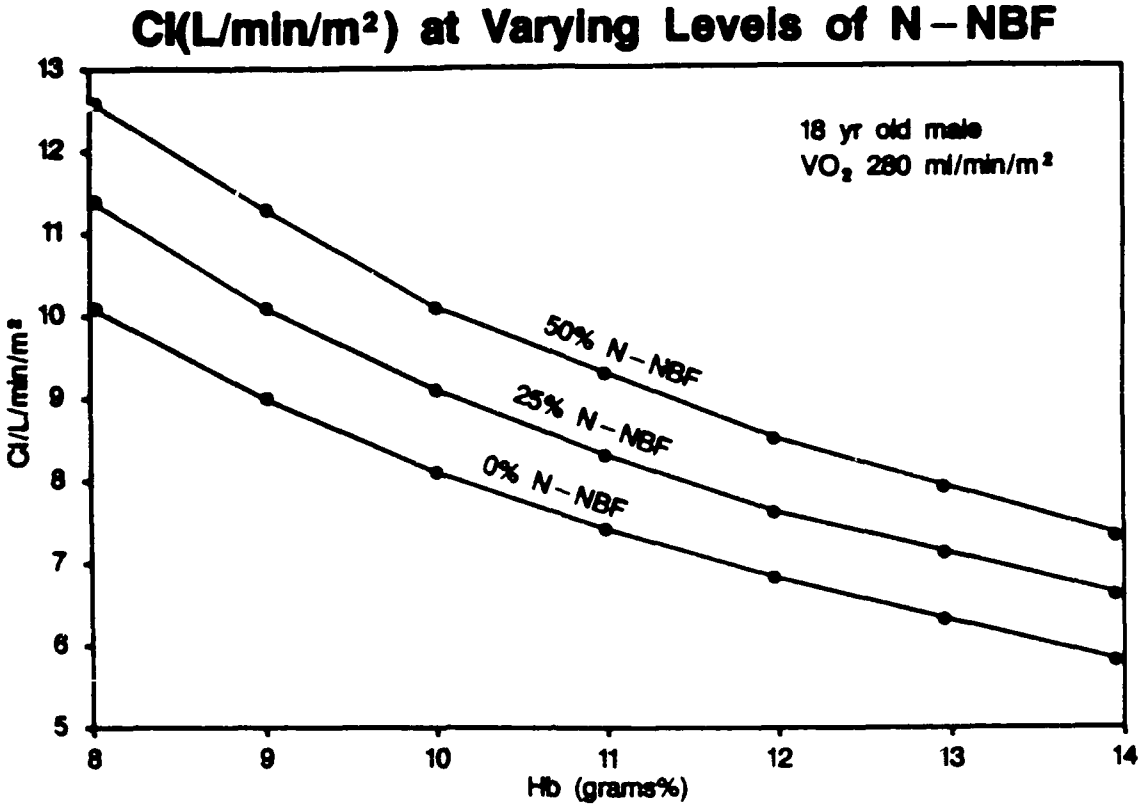

FIGURE 1. Cardiac index (CI) vs. hemoglobin concentration (Hb) with varying degrees of nonnutrient blood flow (NBF) in an 18-yr old male with normal arterial oxygenation and a twice normal oxygen consumption.

These relationships should be considered during the care of hypermetabolic burn patients. It is suggested that normalizing Hb, i.e., 12-14 g %, may be hemodynamically advantageous. This would be especially important in those patients with poor cardiac reserve.

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SUMMARY OF PART IV - MISCELLANEOUS

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THE PRECEDING papers intellectually traverse the spectrum of burn care from the physiology of acute injury to rehabilitation. All of them raise provocative issues that warrant further investigation and fuller elucidation.

For instance, 50% of all burn deaths nationally occur within the first 5 days postinjury, reflecting our imprecise understanding and/or treatment of shock and inhalation injury. Dr. Farrell's study of hemoglobin concentration and cardiac output makes one wonder why so little research has been devoted to this problem. Many physicians consider a hematocrit of 34 reasonable, as if they were treating patients with chronic medical problems. While a hematocrit of 27 may be desirable for the postcardiac bypass patient, a hypermetabolic burn patient is surely different. Though teleologic reasoning is always questionable, millions of years of evolution would indicate that a normal hemoglobin of 14-15 assures optimum organ perfusion. The high nonnutrient blood flow in our burn patients would suggest keeping the hemoglobin increased even when the patient is not septic. If the skin is the injured organ at risk and oxygen is a rate-limiting step in wound healing, then perfusion is necessary to insure healing of 2° burns or skin grafts.

Dr. Welch's paper appropriately emphasizes the pitfalls for anesthesiologists and the complexities of care with the burn patient. Research proceeds in how to better control temperature lability in the anesthetized patient in the operating room and, perhaps most importantly, pain control. Pain from onset of injury to healing remains a major challenge for both staff and patients. All presently used agents have the side effects of nausea (increased caloric intake is necessary), respiratory inhibition (when atelectasis and pneumonia are genuine threats), or, worst of all, addiction.

Histologic classification of burn wound infection is even more significant for what it implies rather than what it says. Quite simply, the clinical team effort in 1988 relies heavily on laboratory support. Too many institutions lack pathologists skilled in rendering the diagnosis of burn wound infections discussed in Dr. Kim's paper or technicians skilled in preparing the slides. The evolution of the present system as well as the employing of quantitative microbiology have taken several decades and warrant further reading. The uninitiated should specifically be aware of the significance of swab cultures of the wound, the rapid slide technique, and

conventional microbiology from wound biopsies versus the pathology described in this paper.

Dr. McManus' paper reads like a good detective yarn and in fact once again emphasizes the importance of environmental factors in infection. Contrary to the belief of rotating residents, cross-contamination is not inevitable, but once resistant strains are in place, it may take the radical measures illustrated here to eradicate them.

The significance of Dr. Larson's work has been to make us realize that hypertrophic scars are not automatic sequelae of burns and that their occurrence can be reversed by mechanical means. The full biochemical explanation of this work is yet to be unraveled and one suspects that pharmacologic manipulation of these wounds will be the next major breakthrough in scar treatment. More significant than the scientific contribution, however, has been Dr. Larson's sensitivity to outcome and rehabilitation of these unfortunate patients.

CHAPTER V - IMMUNOLOGY AND HOST RESISTANCE

Two major events occur when the complement system is activated by IgG and bacteria. First is the process of opsonization which is caused by the deposition of IgG and C3b on the microbial surface. Phagocytic cells, predominantly neutrophils in man, have recognition sites on their cell membrane for the Fc portion of the IgG molecule (the Fc receptor) and for C3b (the CR1 receptor) and its larger degradation product, C3bi (the CR3 receptor). The deposition of these proteins on microbial surfaces, along with the interaction of less specific proteins for which the phagocytic cells may have receptors, is called opsonization. The interaction of the serum proteins and their degradation products deposited on microbial surfaces, with specific ligands and receptors on phagocytic cell membranes, causes binding and subsequent phagocytosis. This process is essential for resistance to infection in man.

A second major consequence of activation of the opsonic system is the generation of a large number of biologically active products which, while helpful in some cases, may be deleterious in others. In particular, there may be activation of the clotting and kinin systems, vasospasm, ischemia, and thrombosis, and the generation of free oxygen radicals.

A study was performed to determine the importance of defects of opsonophagocytosis in 32 seriously burned patients with an average total body surface area burn size of 60.7% and an average 3° burn size of 43.3% (1). Serial tests were performed two times or more weekly, including assessment of the ability of isolated neutrophils from the patient to ingest and kill bacteria, performance of an opsonic index, and a measurement of certain opsonic proteins, including C3, IgG, properdin, and Factor B. The ability of neutrophils to kill bacteria and the opsonic index were both depressed in this group of patients. In patients who had a mild or moderate degree of neutrophil dysfunction, the degree of abnormality of the opsonic index could not be correlated with the incidence of bacteremia or with the number of episodes of bacteremia per patient. In contrast, in patients with relatively severe disorders of neutrophil function, worsening of the opsonic index was associated with an increased incidence of bacteremia and the number of bacteremic episodes per patient. These findings were interpreted, probably incorrectly, as evidence that opsonic abnormalities were an important factor in depressing resistance to bacteremia in seriously burned patients, but only when there was an associated severe abnormality of neutrophil function. These and other studies in seriously burned patients have shown that opsonic index often has a significant and striking fall, often to 20-30% of normal values, and this is usually paralleled by a fall in serum C3. However, following the first few days after burn injury, the C3 tends to rise, sometimes to 2 or 3 times normal values, with an increased accumulation of degradation products in the serum.

To further define the importance of low levels of opsonic capacity in the blood, a study was done in a canine model which involved massive hemorrhage and resuscitation of the animals with either packed red blood cells or whole blood (2). In the animals resuscitated with packed red blood cells and electrolytes, opsonic activity dropped to approximately 20% of normal values with a 50-60% reduction in IgG and C3. However, viable organisms injected either intravenously or subcutaneously were cleared at a normal rate. In another study, cobra venom factor was given to guinea pigs to cause almost complete depletion of serum opsonic activity; these animals showed a corresponding, striking reduction in the ability of their sera to opsonize bacteria. Furthermore, serum replacement after opsonic depletion by cobra venom factor restored opsonic activity to the serum. Both serum-replaced and nonserum-treated animals were given an intraperitoneal challenge of Escherichia coli and it was found that serum-treated animals had a better survival. The opsonic activity associated with survival approximated 10% of the opsonic activity of normal serum. In dilution studies using normal serum, most bacteria are completely opsonized by dilutions of 10% or more, often as low as 1% serum concentration.

From these studies, we have concluded that defects of opsonic activity in the serum of patients is a frequent occurrence, but there is a large functional reserve. To be clinically significant, reductions of 90% or more in nonspecific serum opsonic activity is probably necessary, situations rarely found in man even after severe burn injury. It is not surprising then that a prior study, in which fresh frozen plasma was administered to seriously burned patients, showed no clinical benefit when compared to a group of patients given equivalent amounts of albumin (3). It is also consistent with the finding that the passive administration of large amounts of intravenous gammaglobulin to seriously burned patients is of little clinical benefit (4).

In recent years, complement degradation products have been found to have an important effect on phagocytic functions (5). As an example, C3a induces histamine release from basophils and may, under certain conditions, induce primary and secondary granule enzyme release from neutrophils. C3b, as noted earlier, induces phagocytosis when membrane-bound. Fluid phase C3b, however, can inhibit phagocytosis and killing of bacteria, stimulate the production of active oxygen species, and induce the release enzymes. C5a also induces secretion of lysosomal enzymes from neutrophils and macrophages, may cause nonspecific deactivation of neutrophils, aggregrates neutrophils, enhances random migration, induces neutropenia, and induces the release of interleukin from macrophages and thromboxane from activated platelets. These complement products may accumulate following burn injury because of an increased rate of synthesis, increased activation, and decreased levels of inhibitors.

Fluid phase C3b is a particularly important degradation product, although specific levels have not been measurable in man due to the lack of suitable monoclonal antibodies. However, C3b can be isolated from normal human serum and occurs in elevated levels in patients with burns and traumatic injury (6). In in vitro studies, purified C3b has a profound inhibitory effect on the opsonophagocytosis of bacterial species that utilize the alternative pathway of complement during opsonization. This appears to occur because of interference with both the CR1 and CR3 receptor sites (7). More importantly, when fluid phase C3 was injected intradermally with bacteria, lesion sizes were increased compared to bacteria injected with either saline or albumin (8). An even more striking effect was noted when bacteria was introduced intraperitoneally with C3b. Clearance of microbes from the peritoneal site was markedly inhibited. Thus, fluid phase C3b inhibits the phagocytic capability of neutrophils, and this may be clinically important in situations where trauma and/or sepsis causes large amounts of C3 to be produced and activated.

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INFLAMMATORY MEDIATORS

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IN RECENT years, it has become clear that the microcosm of the individual cell is finely regulated by a diverse array of chemical mediators. Homeostasis is maintained by continual interaction of cells, both under normal conditions and under circumstances of minimal perturbation of the cells. However, the consequences of injury of greater magnitude can be observed as a local as well as a systemic effect of an exaggerated response. Although refinements in the approach to resuscitation and early medical intervention have led to prolonged survival in the acute phase after injury, the systemic response to injury has now been incriminated in delayed mortality. Since it is likely that the acute phase response to injury contributes to delayed pathophysiology, current studies are directed at modifying approaches to resuscitation and later care based on the recognition of the deleterious effect of uncontrolled inflammatory mediator expression. The present review deals with the general aspects of expression of mediator activity, sources of mediators, and the evidence that inflammatory mediators participate in the pathophysiology of injury-induced perturbation of homeostasis.

Expression of Mediator Activity. A mediator system participates in the stimulus-induced response when activation is followed by generation or release of an active substance in sufficient quantity or concentration to lead to an effect on a secondary cell or pathway. The expression of mediator activity is, furthermore, dependent on modulation of its intrinsic activity as well as of the target cell or pathway. From this standpoint, it is often difficult to relate *in vitro* studies to the complex interactions that occur *in vivo*. For example, *in vitro* data indicates that mast cell activation can occur via mechanisms that are initiated by injury. These data also indicate that the mediators histamine, heparin, chemotactic factors, and proteolytic enzymes are released from the cell as a consequence of activation. However, the activity of each mediator may be modulated by specific inactivators or inhibitors (1). Thus, *in vivo* histaminase may inactivate histamine and heparin and proteolytic enzyme may mutually inhibit expression of activity. In addition, the target of the mediator may be rendered unresponsive as in the case when elevated intracellular levels of cyclic adenosine monophosphate diminish cellular responsiveness to receptor-mediated activation. Therefore, the expression of a mediator system is dependent on complex interactions that, in the final analysis,

are much better evaluated in vivo. A majority of the data that supports a role for various mediator systems in the development of pathologic changes after injury is dependent on in vivo measurement of mediators or their stable metabolites or on methods that selectively block mediator release, generation, or expression. These approaches are limited by inability to detect mediators which often act at the cellular level at nano- to femto-molar concentrations.

Sources of Mediators. Inflammatory mediators may be generated or released from plasma cascades or cells. The plasma cascades include the coagulation, complement, and angiotensin systems. Each system is composed of a series of proteins or peptides that enzymatically activate the pathways in sequence. Initiation of the coagulation pathway by activation of Hageman factor (Factor XII) can lead to coagulation, kinin generation, and fibrinolysis. The active products of the complement pathway include the anaphylatoxins, C3a and C5a, which cause mast cell degranulation. In addition C5a is a potent stimulus for neutrophil chemotaxis and degranulation. Activation of the complement components, C7, C8, and C9, can cause cell lysis and the fragments of C3 have been shown to modulate neutrophil responsiveness. Angiotensin II, a potent vasoconstrictor, is generated by the action of renin (which may be generated from prorenin by cleavage by kallikrein of the coagulation pathway) on angiotensinogen. The intermediate polypeptide production, angiotensin I, is further cleaved by angiotensin-converting enzyme to yield angiotensin II. An enzyme of the neutrophil, cathepsin G, can cleave angiotensin II directly from angiotensin.

Cells that may generate mediators during the inflammatory response are macrophages, mast cells, lymphocytes, polymorphonuclear neutrophils, and endothelial cells. A common denominator in most systems studied appears to be initiation of arachidonic acid metabolism by membrane perturbation. Although some cell lines such as macrophages generate products of both the cyclooxygenase (prostaglandin) and lipooxygenase (leukotriene) pathways, others appear to predominantly produce products of one pathway. For example, neutrophils primarily metabolize arachidonic acid to leukotriene B_4 whereas mast cells produce more prostaglandin products. The specific inflammatory mediators of the mast cell have been previously mentioned and reviewed (1). The plethora of mediators derived from the macrophage have recently been reviewed (2) and include interleukin 1 and tumor necrosis factor.

Mediators and Postinjury Pathophysiology. Mediators such as histamine (3) have been shown to be released into the plasma after burn injury in quantities sufficient to cause increases in vascular permeability and hypotension in animal models. Chemotactic factors, when present at concentrations below the level of detection, have been shown to increase susceptibility to infection after burn injury (4). Additional data suggest

that the increased susceptibility to infection after injury may be a consequence of increased but inappropriate activation of neutrophils (5) and such has been suggested to participate in organ failure at distant sites after injury. Recent data such as that concerning interleukin 1 and tumor necrosis factor suggest that the macrophage may participate in the pathophysiologic changes that follow injury. This is of particular interest from the viewpoint of the growing awareness of the role of the gastrointestinal tract as a source of systemic hormones, endotoxin, and bacteria. Nevertheless, it is not possible at this time to implicate only one pathway as primary in the response to injury.

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BURN-RELATED HUMORAL IMMUNOSUPPRESSANTS

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BURN LITERATURE is replete with convincing and experimental evidence documenting the acquired immunological depression that occurs within 24 h of major thermal injury. Moreover, specific cellular abnormalities can be returned toward normal when a particular cell is removed from the burn environment and placed into a more normal environment such as fresh frozen plasma or donor blood. It is clear that the internal milieu of burn patients contains circulating factors that are toxic to cellular function and presumably at least partially responsible for the postburn immunosuppression. Evidence from many laboratories has implicated both exogenous and endogenous factors in the systemic circulation as possible causes of postburn immunosuppression. These include vasoactive amines, serotonin, prostaglandin/leukotrienes, bacterial endotoxin, neutrophil products, complement activation products, protein breakdown products, metabolic hormones such as corticosteroids, catecholamines, serum proteins such as immunoglobulin, immune complexes, α-globulins and α-keto-proteins, and iatrogenic substances such as antibiotics, topical chemotherapeutic agents, anesthesia, and multiple transfusions.

The classic diagram of wound healing by Hunt demonstrates inflammation as the initial process following trauma. Inflammation has been defined since Celsus in the first century with rubor (vasodilation of the blood vessels), tumor (increase in the permeability of blood vessels), calor (increased blood flow to the inflamed area), and dolor (stimulation of the sensory nerve endings in the changed tissues). In 1920 Sir Henry Dale defined the criteria for inflammatory mediator to be as follows: the proposed mediator must occur in appropriate concentration at the site of inflammation and enzymes for synthesis and breakdown must also be present, the proposed mediator must reproduce all of the biological features observed during inflammation, and pharmacological manipulation of the synthesis release and/or action of mediator should all alleviate the symptoms of inflammation.

Many inflammatory mediators have been described at the site of injury and systemically following burn injury. A possible hypothesis for the immunological alterations following thermal injury would be an uncontrolled systemic inflammatory response with the control of feedback mechanisms for the local inflammatory response, resulting in systemic immunosuppression.

The following is a summary of the inflammatory mediators that have been described following thermal injury. Yurt and

others demonstrated that plasma histamine concentration became elevated within 2 min following burn injury in an animal model. Moreover, this elevation was proportional to the burn size, with a higher and prolonged level being present after a 60% total body surface area burn in the rat model. In addition, Hansbrough demonstrated in a mouse model that an H_2 antagonist, cimetidine, ameliorated the delayed hypersensitive response of ear swelling. The acute inflammatory mediator, serotonin, was evaluated in a pig model with the use of a specific blocking agent, ketanserin. The depressed cardiac and pulmonary hypertension normally seen following major thermal injury was not seen in the animals receiving ketanserin.

The participation of arachidonic acid metabolizing inflammation has been studied extensively and has been demonstrated to be elevated following thermal injury. Remarkably, the arachidonic acid metabolites fit the criteria of Sir Henry Dale as inflammatory mediators, with production of heat via the cyclooxygenase pathway, redness and swelling caused by vasodilation of the endoperoxidase, PGE, PGE_2, PGD_2, and PGA_2. In addition, most arachidonic acid products induce hyperesthesia, pain due to synergy with bradykinin and histamine. The regulation of the immune response by prostaglandin has been well demonstrated to be involved in the delayed hypersensitivity response. Herndon demonstrated that burn patients had normal keto-PGF levels in both major burns and burns with sepsis; however, he demonstrated a marked elevation in thromboxane during the first 3 days following thermal injury and in septic burn patients.

The inflammatory mediator, interleukin 1, with its multiple functions, including fever, neutrophilia, activation of neutrophils, collagen proliferation, amino acid release, release of acute-phase proteins, activation of T lymphocyte production via interleukin-2, and activation of B lymphocytes to produce antibody has been demonstrated by Mannick to be elevated following thermal injury.

Ninnemann and others have extensively studied an immunoglycoprotein. This protein has an ion molecular weight between 1000 and 5000 amu. It has a complex composition containing protein, carbohydrate, and lipid. It is heat-stable and is unaffected by enzymatic digestion and is very immunosuppressive. Its suppressive mode of action depends upon the presence of arachidonic acid metabolites.

Circulating burn toxins have been demonstrated for many years beginning in 1919 by Cannon and Bayliss, who proposed the theory of burn toxins to account for late deaths. Rosenthal demonstrated inhibition of cultured cells blocked by convalescent burn serum in 1937. In 1983, Dobrokovski and Frendorov demonstrated burn treatment with convalescent serum.

Fox demonstrated thromboplastic action from extracts of skin in 1965. In 1968, Baxter demonstrated myocardial depression factor presentin the serum following burn injury. Schoenenberger and others demonstrated a burn toxin that causes multisystem organ failure in an animal model. Whether this burn toxin is a multitude of circulating mediators remains to be seen; however, endotoxin has been demonstrated to be present in the burn serum within 24 h following thermal injury and the endotoxin caused suppression of the mixed lymphocyte response. In addition, this response could be substantiated in human volunteers who, after a small dose of endotoxin, had marked suppression of lymphocyte function.

The proteolytic amplification cascades, which are systems characterized by highly specific consecutive proteolytic reaction modulated by plasma protease inhibitors such as C1 inhibitor, α_2-antiplasmin, antithrombin, and α_2-macroglobulins, are also altered following thermal injury. The protease amplification cascade systems include the kinogen, complement, clotting, and systems. Holder demonstrated a marked elevation in proteolytic activity following major thermal injury, and this elevation appeared to be related to burn size. The correlation between the proteolytic cascades is very complex and only partly understood. Complement activation has been demonstrated following thermal injury, and Mannick and Moore demonstrated neutrophil activation via complement receptors in 7 patients with burns.

The classes of inflammatory mediators may be divided into two major groups. The first group are vasopermeability mediators which include vasoactive amines such as serotonin and histamines, peptides such as bradykinin and luekokinins, and prostaglandins. The second class of inflammatory mediators are chemotactic mediators which include prostaglandins, the peptide C5a, neutroproteases, and leukokinsis-enhancing factor. All of these inflammatory mediators may result with systemic activation neutrophils, resulting in increased neutrophil adhesiveness and formation of leukocyte microemboli, and the depression of leukocyte chemotaxis.

Thus, the postburn immunological alterations mediated by inflammatory mediators result in a chronic activation of the amplification cascade systems, resulting in consumption. The activation of the coagulation fibrolytic system results in increases in levels of fibrinogen, fibrinopeptides, and plasmin and a decrease in levels of prothrombin, specific clotting factors, antithrombin III, and plasminogen. The platelet count is remarkably increased immediately following thermal injury. The platelets are increased within 48 h following thermal injury. The kinin system demonstrates decreased kallikrein activity. The complement system results in a decrease in opsonic activity, C3, properdin, C3 conversion, and C_{H50} and an increase in C2a, C3a, and Factor B. The products of

arachidonic acid metabolism demonstrate an increase in PGE_2 and leukotrienes. Tissue-derived mediators demonstrate an increase in histamine, serotonin, and neutrophil products. The metabolic hormones, including cortisol and catecholamines, also increase. The hematologic system demonstrates a decrease in the red blood count and increase in the white blood count. Indeed, if the immunological suppression following thermal injury is the result of a systemic inflammatory reaction, we are left with several questions. Is the suppression of cellular function necessary for normal wound healing? In addition, if feedback mechanisms necessary for local inflammation are blocked, will normal wound healing occur and will the inhibitor or "cocktail" blockade result in more harm than good? The understanding of local wound inflammation and wound repair will lead to the understanding of this systemic inflammatory response and the immunosuppression which follows thermal injury.

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EFFECT OF BURN INJURY AND INFECTION ON LYMPHOCYTE POPULATIONS

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CHANGES IN function and phenotype of circulating leukocytes may be related to the defect in host resistance underlying the increased susceptibility of burned patients to opportunistic infection. Among the changes frequently reported in burned patients are a reduced capacity to reject allogeneic skin grafts and impaired response to delayed hypersensitivity antigens. The component of the host defense system responsible for rejecting skin grafts are T lymphocytes. The relationship between this depression of T cell function and susceptibility to opportunistic infection remains unclear.

We have investigated some of the phenotypic changes in the circulating lymphocyte subpopulations of burned patients. Our goals were to assess host defense status, uncover deficiencies in the immune response, and suggest reasonable approaches for corrective therapy.

MATERIALS AND METHODS

Lymphocyte subpopulations were measured in 63 burned patients and 30 unburned controls. All patients in this study were admitted during the first 5 days postinjury and had expected mortalities between 20-90%. The average burn size was 44.5%, the average age was 43.4 yr, and the expected mortality for the group, based on previous experience at this Institute, was 50.9% (1). Peripheral blood samples were obtained twice weekly for up to 8 wk postburn for assessment of cell numbers, subpopulations, and morphology. The controls were sampled at random for comparison. Cells were purified on Ficoll-Hypaque and stained with monoclonal antibodies having specificity for various surface antigens.

The changes in the number and morphology of circulating leukocytes following thermal injury are quite dramatic. Shortly after injury, burned patients become leukocytotic and lymphopenic. Most of the cells are granulocytes, many being immature or otherwise abnormal. This combined leukocytosis and lymphopenia make separation of lymphocytes for analysis difficult. Ficoll-Hypaque density gradients separate PMN and RBC from lymphocytes by density, the heavier PMN and RBC settling to the bottom of the tube while the less dense mononuclear cells stay at the blood:gradient interface. In normals, this results in a preparation containing about 90% lymphocytes. In burned patients, however, abnormal PMN with densities similar to lymphocytes significantly contaminate the lymphocyte preparation. To avoid this problem and the

artifacts that might be introduced by extensive physical separation techniques, we used flow cytometry to measure lymphocyte subpopulations. Flow cytometry examines cells individually in a sequential fashion so that each cell can be analyzed independently of all the other cells in the preparation. Lymphocytes can be distinguished from monocytes and PMN by measuring light scatter intensity at forward and 90° angles. Lymphocyte subpopulations were resolved by the addition of subset specific monoclonal antibodies bound to fluorescent dyes and determining the proportion of the lymphocytes binding the monoclonal antibody marker.

RESULTS

The T lymphocyte subpopulations determined in burned patients and controls are shown in Figure 1. The proportion of T cells (as measured by the Pan-T cell markers Leu-1, Leu-4, and Leu-5) as well as the helper and suppressor/cytotoxic subsets (Leu-3 and Leu-4, respectively) were reduced in burned patients. These subpopulations can also be represented as the absolute numbers of cells per unit of whole blood rather than the percent of positive lymphocytes. When compared in this manner, a similar pattern was evident (data not shown). All T lymphocyte subpopulations were significantly decreased in the burned patients compared to controls.

Several non-T lymphocytes markers were also used, i.e., Leu-7 (large granular lymphocytes), Leu-11 (NK cells), Leu-12 (B cells), Leu-M3 (monocytes), and Leu-15 (C3 receptor). As shown in Figure 2, there was no difference in the proportion of non-T-cells found in burned patients and controls when compared either as percent positive cells or as absolute numbers of positive cells per nanoliter of whole blood (data not shown). Since the proportion of T cells decreased and the proportion of non-T cells remained the same, there must have been an increase in "null" cells having none of the common antigens that were tested.

The patients were further divided into two groups based on mortality and compared. Patients who died had decreased T cell subpopulations compared to those who lived (Fig 3). Survivors had decreased T lymphocytes compared to unburned controls, but the nonsurvivors had greater decreases in these subpopulations. There was no difference in non-T-cell subpopulations between the survivors and nonsurvivors (Fig 4).

DISCUSSION

These data have not yet been analyzed with respect to infection, but data on lymphocyte subpopulations during infection are available from the burned rat infection model. In contrast to humans, rats with 30-50% burns do not normally develop fatal sepsis, even in the absence of treatment. As a result of the burn injury, they are, however, uniquely

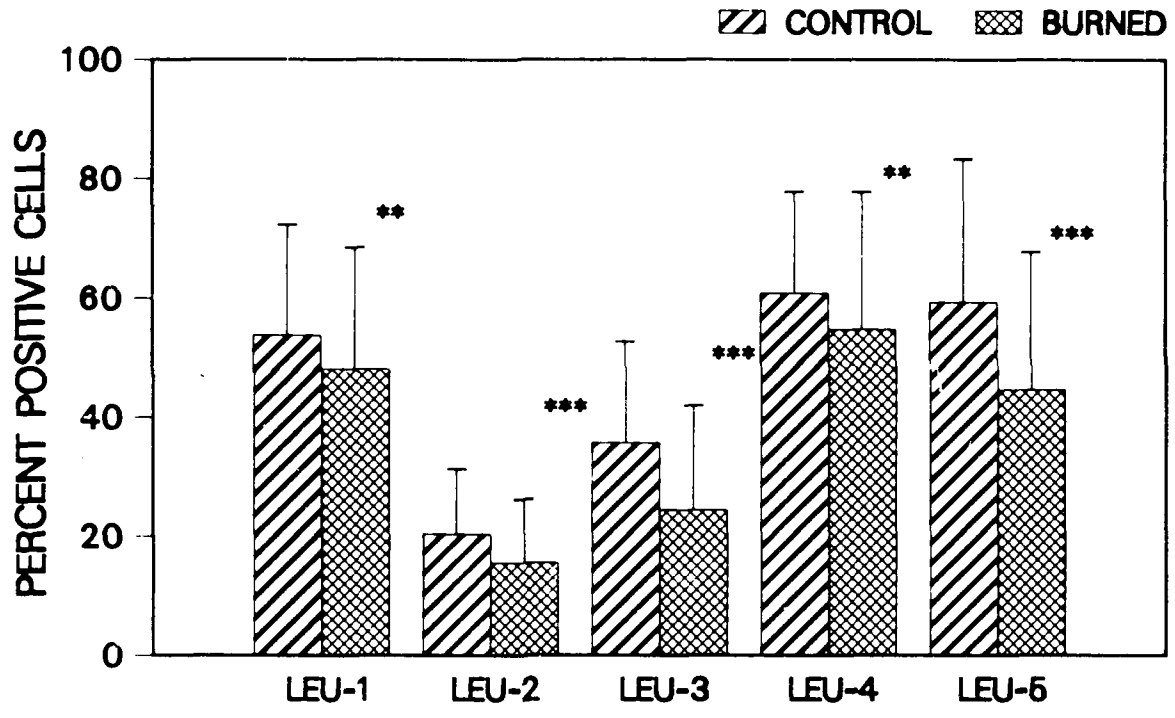

FIGURE 1. T lymphocyte subpopulations from the blood of burned patients and controls. Values shown are mean percent positive cells ± 1SD of controls and patients. *P < 0.05; **P < 0.01; ***P < 0.001.

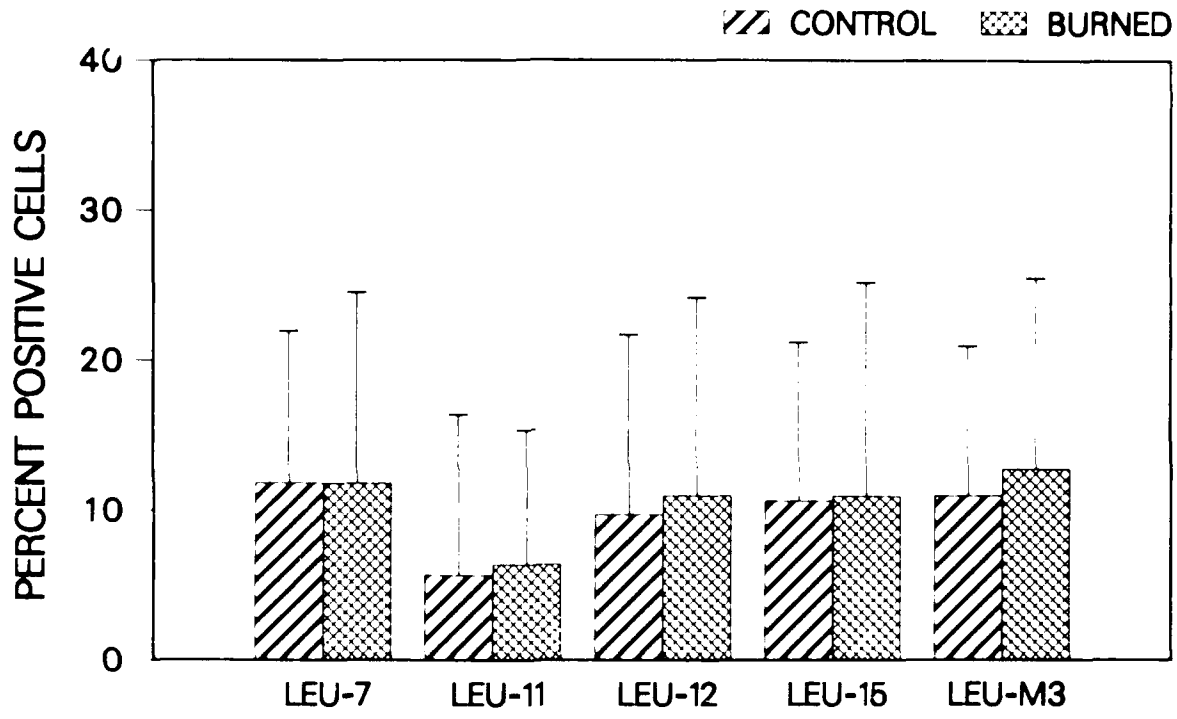

FIGURE 2. Non-T cell subpopulations from the blood of burned patients and controls. Values shown are mean percent positive cells ± 1SD of controls and patients. *P < 0.05; **P < 0.01; ***P < 0.001.

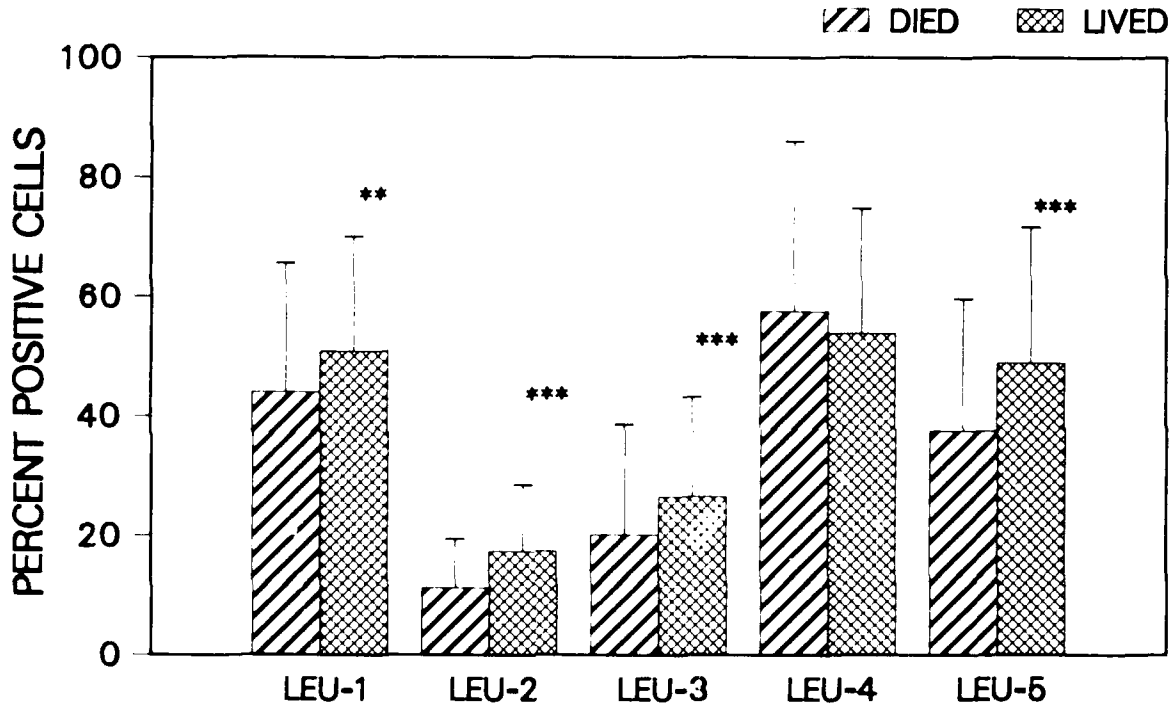

FIGURE 3. Comparison of T lymphocyte subpopulations for survivors and nonsurvivors. Values shown are mean percent positive cells ± 1SD of controls and patients. *P < 0.05; **P < 0.01; ***P < 0.001.

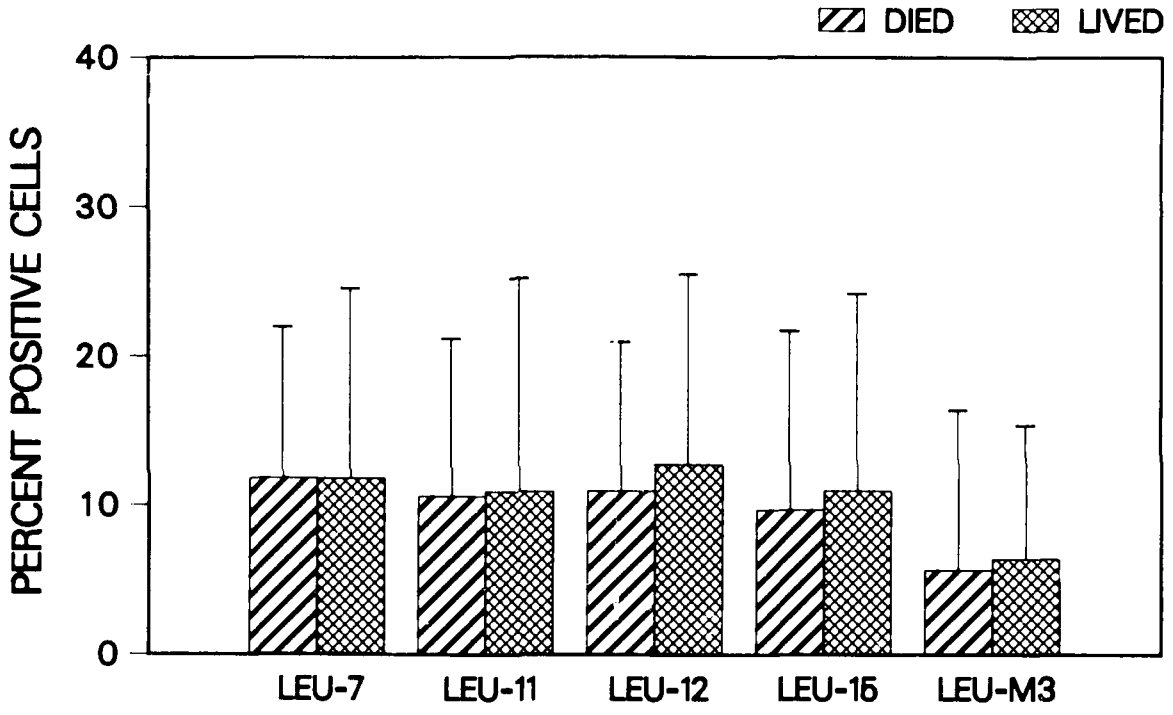

FIGURE 4. Comparison of non-T cell subpopulations for survivors and nonsurvivors. Values shown are mean percent positive cells ± 1SD of controls and patients. *P < 0.05; **P < 0.01; ***P < 0.001.

susceptible to infection by Pseudomonas aeruginosa (Strain 1244) and will die of sepsis if seeded with that organism.

Animals were divided into three groups, i.e., unburned control, burned, and burned with infection. Blood was obtained and the lymphocytes separated on Ficoll-Hypaque. Cells were stained by the appropriate monoclonal antibodies and analyzed by flow cytometry. There were no changes in the T lymphocytes in 30% burned animals. Only after infection was there a decrease in the proportion of the helper cells and a small increase in the proportion of suppressor/cytotoxic cells in these animals. This difference represented a selective depletion since the number of WBC and lymphocytes were severely decreased. The relative shift in the proportion of these subsets caused the ratio of helper cells to suppressor cells to decrease.

The relative proportion of helper to suppressor cells (termed the helper/suppressor ratio) has been proposed as a measure of immunosuppression. Antonacci et al (2) as well as McIrvine et al (3) have reported a decreased helper/suppressor ratio in burned patients compared to normal controls, and the ratio could be related to mortality in one study (3). That study was done with fluorescence microscopy rather than flow cytometry. Both studies were done on substantially smaller patient populations.

The most striking differences observed in our study were a selective decrease in T lymphocytes in burned individuals and a greater decrease in nonsurviving than in surviving patients. As a result, there was an increased proportion of cells of unknown phenotype. It is possible that the decreased cell-mediated immunity and increased infection susceptibility of the burned patients is related to the decreased numbers of T cells in the circulation. The decrease in the number of T cells may decrease the number of functional effector cells able to respond to an infection challenge.

The nature of the null cells is unknown but several possibilities exist:

1. They could be "activated" cells that have shed their normal antigens.

2. They could be nonlymphoid cells contaminating the sample because they that were not eliminated from analysis by the light scatter "lymphocyte window."

3. They could be immature cells that have not developed detectable markers.

4. They could be cells of currently undefined specificity and function.

Whatever their phenotype, they may represent active "suppressors" of the host response or merely cells of limited function that cannot respond appropriately. Although it is important to enumerate the cells of each subset, information about cell function may be more critical to evaluating host defense. Our future efforts will endeavor to measure cell function as well as cell phenotype.

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LYMPHOCYTES AND LIPID PEROXIDATION

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THE GENERALIZED loss of immune function is one of the most serious consequences of severe thermal injury. Lymphocytes from patients with major burns show defects in proliferative responses to T cell mitogens and alloantigens (1-4). This defect correlates with the diminished capacity of burned patients to reject skin allografts and has been associated with a high rate of infection and mortality. In addition, neutrophils from patients with major thermal injuries show defects in chemotaxis and microbial killing. These patients also have elevated plasma C3des-arginine levels and the chemotactic response of their neutrophils to C5a is decreased (5). These findings suggest that thermal injury leads to systemic activation of neutrophils, which renders them unresponsive to subsequent stimuli. In addition, we are hypothesizing that neutrophil activation leads to production of hydrogen peroxide, which subsequently suppresses T cell responses. The combined effects of thermal injury on neutrophil and T cell functions leave the host susceptible to viral, fungal, and bacterial infections. The studies described here were performed to determine if neutrophil activation or hydrogen peroxide could suppress T cell functions.

MATERIALS AND METHODS

Peripheral blood mononuclear cells (PBMC) were isolated from normal whole blood and resuspended in RPMI-1640 supplemented with 10-20% human AB serum as previously described (6). In addition, neutrophils were isolated by dextran sedimentation of whole blood to remove erythrocytes followed by density gradient centrifugation on Ficoll-Hypaque. The response of PBMC to mitogens (phytohemagglutinin (PHA), concanavalin (ConA), and pokeweed mitogen (PWM)) and both proliferation and the generation of cytotoxic T lymphocytes (CTL) in the mixed lymphocyte culture (MLC) were measured as previously described (7-8). The production of hydrogen peroxide (H_2O_2) was measured by the technique of Pick and Keisari using horseradish peroxidase and phenol red (9).

RESULTS

As can be seen in Figure 1, H_2O_2 was found to be a potent inhibitor of human T cell responses. H_2O_2 (100 μM) inhibited PHA, ConA, and MLC responses, and the generation of CTL by

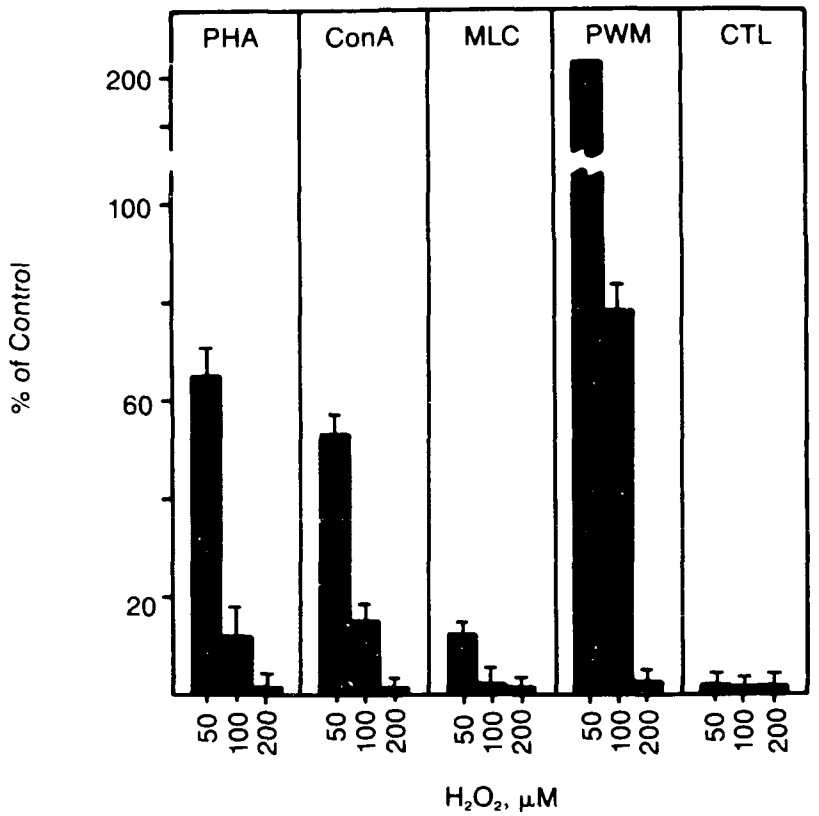

FIGURE 1. Effect of H_2O_2 on in vitro immune responses. H_2O_2 was added to cultures prior to addition of mitogens or allogeneic cells. Cultures were then treated with T-cell mitogens (phytohemagglutinin (PHA) and concanavalin A (ConA), or a B cell mitogen (pokeweed mitogen (PWM)). Cultures were also stimulated by allogeneic cells and assayed for proliferative response (mixed lymphocyte culture (MLC)) and generation of cytotoxic T lymphocytes (CTL). Data are expressed as percent of control (without H_2O_2) responses. From Freed BM, Rapoport R, and Lempert N (7). Figure used with permission from the authors.

>90%. In contrast, H_2O_2 had a much different effect on B cell proliferation in response to PWM. Low doses of H_2O_2 (50 μM) stimulated B cell proliferation, although high doses (200 μM) suppressed this response as well. These concentrations of H_2O_2 did not cause cell death.

The effect of H_2O_2 on T cell responses appeared to be directed towards early activation events, since the addition of

H_2O_2 only minutes after stimulating the cells with PHA resulted in a near-complete loss of inhibitory activity (Fig 2). Furthermore, the inhibitory effect of a single dose of H_2O_2 was spontaneously reversed after 48 h (Fig 3), suggesting that the cells were either capable of repairing whatever components had been damaged or that the delayed proliferation was due to a resistant subset of T cells. However, the fact that repeated doses of H_2O_2 resulted in continued suppression suggests that resistant clones of T cells do not exist.

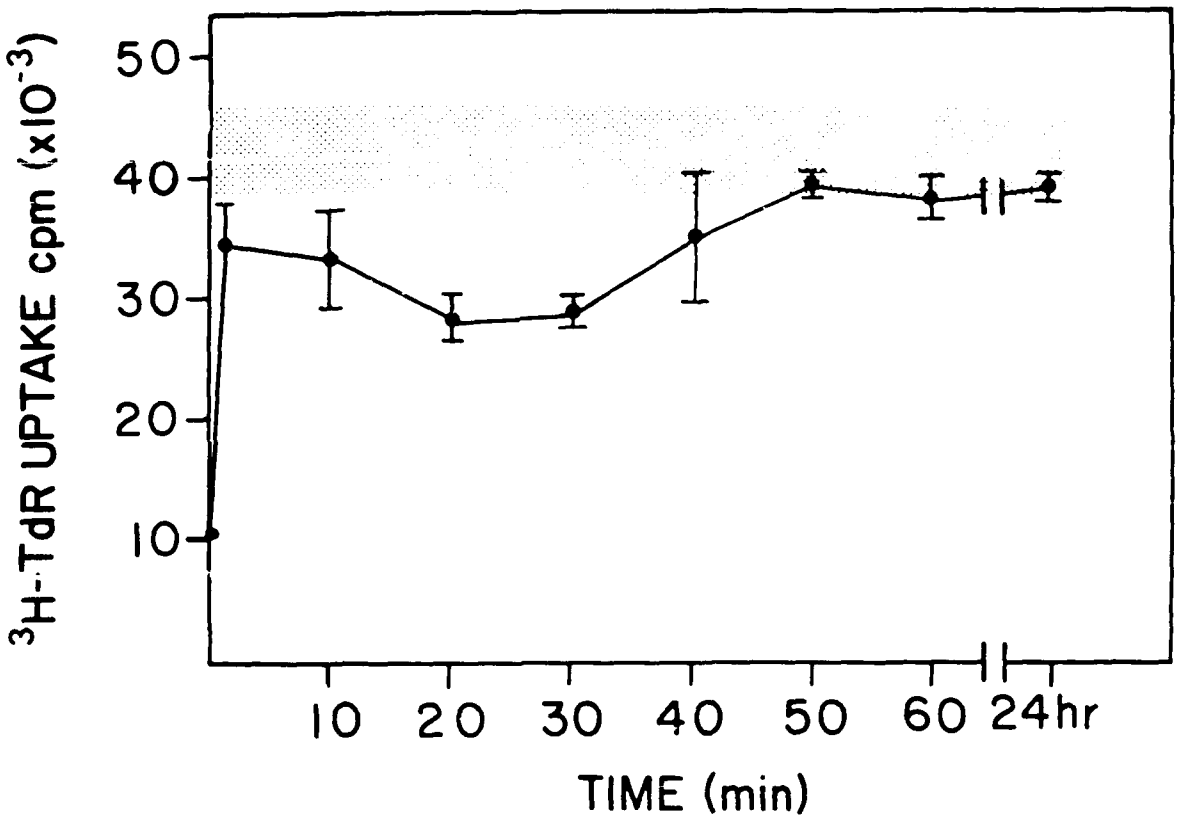

FIGURE 2. H_2O_2 inhibition of early event in T cell activation. H_2O_2 (200 μM) was added before or at various times after addition of PHA. Data are expressed as mean $\pm$ SD. Shaded area represents mean $\pm$ SD of control response. ^{3}H-TdR indicates tritiated thymidine; cpm indicates counts per minute. From Freed BM, Rapoport R, and Lempert N (7). Figure used with permission from the authors.

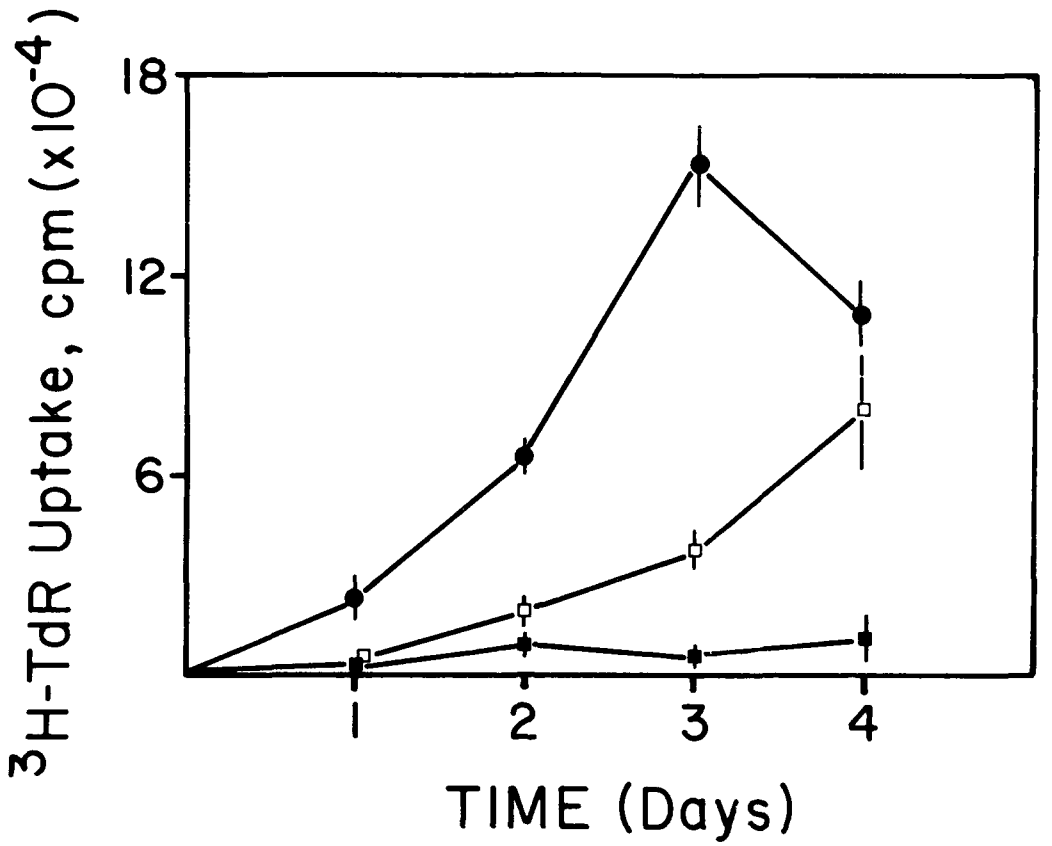

FIGURE 3. Effect of single and multiple doses of H_2O_2 on the kinetics of PHA responsiveness. H_2O_2 (200 μM) was added at time T=0 for the single exposure (□) and additionally at 24-h intervals on days 1-4 for multiple exposures (■). The data are expressed as the mean ± SD of tritiated thymidine uptake (^{3}H-TdR). cpm indicates counts per minute. From Patterson DA, Rapoport R, Patterson MAK, et al (11). Figure used with permission from the authors.

DISCUSSION

The mechanism by which H_2O_2 suppresses early T cell activation events is unknown. We considered three possibilities (Fig 4). H_2O_2 is membrane-permeable and could enter the cell and oxidize cellular thiols that are critical for early activation events (10). However, we observed no change in the total cellular pool of thiols following treatment of PBMC with 200 μM H_2O_2 (7). In addition, H_2O_2 has been shown to induce deoxyribonucleic acid (DNA) strand breaks (through the formation of OH· or lipid radicals), which might result in

suppression of T cell proliferation. However, the fact that H_2O_2 is much less inhibitory when added only a few minutes after T cell activation makes it unlikely that DNA damage is the primary cause of the inhibitory effect. We also postulated that H_2O_2, following a one-electron reduction to OH· via the Haber-Weiss reaction, could oxidize membrane carbohydrates or lipids that are critical for activation. Oxidation of membrane carbohydrate moieties by sodium periodate has been shown to alter T cell responses via the generation of aldehydes, and the effect can be reversed by treating the cell with sodium borohydride. However, we noted that sodium borohydride did not reverse the inhibitory effects of H_2O_2 (7). In contrast, the lipid antioxidants butylated hydroxyanisole, butylated hydroxytoluene, and n-propyl gallate blocked over 80% of the inhibitory effects of H_2O_2. Other lipid antioxidants, including bis-N-methylacridinium nitrate, lucigenin, and vitamin E, were less effective. 2-Mercaptoethanol, which reacts with both free oxygen radicals and lipid radicals, was also able to prevent the inhibitory effect of H_2O_2, provided it was added prior to the oxidant (Table 1). Although the model shown in Figure 4 suggests that the generation of the OH· occurs outside the cells, we have found that desferrioxamine (an iron chelator) does not decrease the inhibitory effects of H_2O_2 and the addition of exogeneous iron does not increase the inhibition (11). Thus, we postulate that if the generation of OH· and the induction of lipid peroxidation are involved in H_2O_2-mediated inhibition, they must occur inside the cell.

The effect of H_2O_2 on human T cell responses could be mimicked by the addition of activated neutrophils. As can be seen in Table 2, the addition of 4×10^5 neutrophils, which produced only 13.6 μM/h of H_2O_2, suppressed the ConA response of 2×10^5 PBMC to the same extent as 50 μM H_2O_2 added exogeneously. The inhibitory effect of this relatively small dose of H_2O_2 could be due to its production in close proximity to the lymphocytes, resulting in a much higher local concentration of H_2O_2.

The implications of this research in the management of thermal injuries are evident when one considers that lipid peroxidation, once initiated, is autocatalytic. Hence, early intervention following oxidative insult may be necessary to prevent suppression of cellular immunity. Lipid peroxidation products have been found in the plasma of patients who suffer from major thermal injuries (12). In addition, neutrophil activation and oxygen radicals have been clearly associated with thermally induced acute lung injury (5,13). Thus,

TABLE I. Effect of Lipid Antioxidants on H_2O_2-Mediated Inhibition

Treatment	PHA Response (Mean ± SD)
Control	26744 ± 1341
200 μM H2O2	408 ± 272
10 μM BHA/BHT/NPG	25772 ± 1496
H_2O_2 + BHA/BHT/NPG (0.01 μM)	20772 ± 1966
H_2O_2 + BHA/BHT/NPG (0.1 μM)	20458 ± 242
H_2O_2 + BHA/BHT/NPG (1.0 μM)	17143 ± 889
100 μM NMN	23596 ± 1478
H_2O_2 : NMN (1 μM)	14889 ± 2005
H_2O_2 + NMN (10 μM)	15830 ± 2607
H_2O_2 + NMN (100 μM)	16492 ± 2932
10 μM 2-ME	25216 ± 2296
H_2O_2 + 2-ME (10 μM) at 0 min	22113 ± 3429
H_2O_2 + 2-ME (10 μM) at 10 min	207 ± 1515

*H_2O_2 indicates hydrogen peroxide; PHA, phytohemagglutinin; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; NPG, n-propyl gallate; NMN, bis-N-methyl acridinium nitrate; and 2-ME, 2-mercaptoethanol. All cultures were stimulated with 250 mg/l of PHA and proliferative response was measured on day 2. Expressed as counts per minute of tritiated thymidine. From Freed BM, Rapoport R, and Lempert N (7). Table used with permission from the authors.

prevention of neutrophil-induced oxidative damage may alleviate both the lung injury and T cell defects associated with thermal injury, thereby increasing the patient's resistance to fungal and viral infections. However, unless ways can be found to prevent the systemic activation of neutrophils by complement split products, the defects in neutrophil function may persist and leave the patient susceptible to bacterial infections.

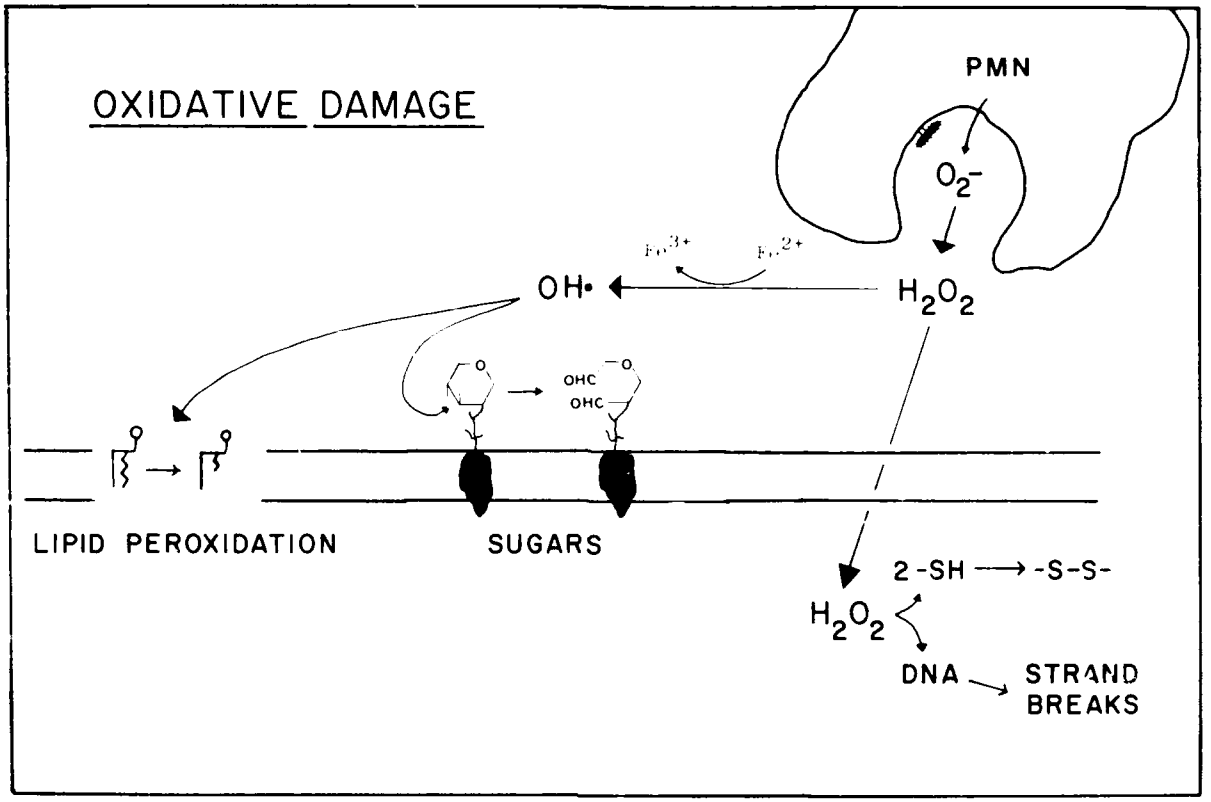

FIGURE 4. Possible sites of oxidative damage to lymphocytes. PMN indicates polymorphonuclear neutrophils; H_2O_2, hydrogen peroxide; DNA, deoxyribonucleic acid.

TABLE II. Effect of Activated Neutrophils on the Response of Peripheral Blood Mononuclear Cells (PBMC) to Concanavalin A (ConA)

Treatment	ConA Response (Mean ± SD)
2 X 10^5 PBMC	78,113 ± 557
2 X 10^5 PBMC + 50 μM H_2O_2	26,016 ± 1,749
2 X 10^5 PBMC + 4 X 105 activated neutrophils*	23,448 ± 2,708

*Neutrophils produced 13.6 nmoles H_2O_2 (13.6 μM) per hour.

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EFFECTS OF INJURY AND SEPSIS ON NEUTROPHIL FUNCTION

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FOR THE PAST 17 yr, 7 of which were spent in association with the US Army Institute of Surgical Research at San Antonio, Texas, my research has been principally concerned with the role of molecular oxygen in immune defense. As a first consideration, oxygen chemistry might appear unrelated to burn care. However, survival of burn patients, as well as other immunocompromised patients, is in large part dependent on the information-effector mechanism provided by the humoral-phagocyte axis of acute immune defense. Oxygen is required for effective phagocyte microbicidal action, and light is emitted as a product of the resulting oxygenation reactions (1). As such, measurement of this chemiluminescence provides a sensitive approach to investigating the acute immune system and its role in protecting normal and immunocompromised patients from infection (2-4).

Quantum Considerations of Oxygen Reactivity. Molecular oxygen reacts exothermically with essentially all organic molecules. Therefore, it might seem paradoxical that we survive and even require an atmosphere that is >20% oxygen (O_2). This apparant paradox is resolved by considering O_2 reactivity in terms of quantum mechanics and absolute reaction rate theory.

In keeping with Hund's rule of maximum multiplicity, O_2 has a triplet multiplicity ground state and a paramagnetic, diradical character. On the other hand, biologic and organic molecules are typically nonradical, diamagnetic molecules of singlet multiplicity. The direction of a reaction is defined by its exothermicity, but factors affecting the actual rate of a reaction are best appreciated through the absolute reaction rate theory approach. Accordingly, reaction rate is defined by the equation:

$$\text{Rate} = x(kT/h)(F\ddagger/FaFb)e^{-Ea/RT}$$

where x is the transmission coefficient, kT/h is the frequency factor (k is the Boltzmann constant, T is the absolute temperature, and h is Planck's constant), and F‡/FaFb is the partition function for activated state with Fa and Fb being the initial states of the reactants. Ea approximates the experimental energy of activation described by the classical Arrhenius relationship and R is the gas constant. As such, rate is influenced by the equilibrium condition of the reactants, the energy of activation, and the transmission coefficient. Not all reaction complexes possessing the

required energy to cross the reaction barrier will do so. The transmission coefficient defines this relationship, i.e., x is the ratio of the reaction complexes that yield product to the total number having reached the activated state (5-7).

Appreciation of the meaning of x is essential to understanding the unique biochemistry of O_2 and its role in microbicidal action. Such an appreciation can be gained from the perspective of quantum mechanics. According to the Wigner spin conservation rule, a reaction system will resist any change in spin angular momentum, i.e., multiplicity. In keeping with the Wigner conservation rule, the value of x becomes very small, typically $<10^{-4}$, for reactions involving change in multiplicity. Thus, the Wigner rule and the related absolute reaction rate expression provide the fundamental explanation why singlet multiplicity biological molecules are protected from direct oxygenation by triplet multiplicity O_2 (4).

Oxygen Activation by the Phagocyte. The microbicidal action of phagocytes, especially the polymorphonuclear leukocyte (PMNL) of the acute inflammatory response, is metabolically driven and O_2-dependent (8). These phagocytes generate the energy and have the enzymatic mechanisms for overcrowding the reactive limitations imposed on O_2 by Hund's maximum multiplicity rule. Respiratory burst metabolism provides the reducing equivalents and potential for univalently reducing O_2 from triplet to single multiplicity, i.e., reduction to perhydroxylic acid ($\cdot O_2H$) and its conjugate base, the superoxide anion ($\cdot O_2^-$). It is probable that univalent reduction of O_2 involves reaction with the riboflavin semiquinone of a flavoprotein oxidase. As such, superoxide was proposed as a product of nicotinamide-adenine dinucleotide phosphate oxidase (1) before its presence was demonstrated by superoxide dismutase-inhibitable cytochrome C reduction (9). One equivalent reduction lowers the multiplicity of O_2. It also decreases its paramagnetic and radical character. In addition, the product acid, $\cdot O_2H$, has a negative log of dissociation constant of 4.8 and may be essential to acidification of the phagolysosome (10).

The acid disproportionation of superoxide is maximum at a hydrogen ion concentration of 4.8, the value of the negative log of dissociation constant, i.e., radical-radical annihilation to yield nonradical hydrogen peroxide (H_2O_2) becomes maximum once the limitation imposed by anionic repulsion is removed. This reaction is a doublet-doublet annihilation and, in accordance with the Wigner-Witmer rules,

should proceed through a singlet reaction surface to yield the singlet multiplicity product H_2O_2 (4).

The microbicial potential of H_2O_2 is fully realized by myeloperoxidase. This enzyme, a major component of the PMNL azurophilic granules, is an H_2O_2:halide oxidoreductase yielding singlet multiplicity HOCl as product (11-12). Further reaction of HOCl with H_2O_2 via a singlet reaction surface yields singlet multiplicity O_2 ($^1O_2$). H_2O_2, HOCl, and $^1O_2$ are of singlet multiplicity and as such, if nonspin symmetry considerations are met, the oxidizing agents can react with singlet multiplicty biologic molecules. When reaction yields dioxygenated intermediates, such as dioxetanes and endoperoxides, there is the possibility for rearrangement, yielding n,π carbonyls that can relax by photon emission. As such, light or chemiluminescence (CL) is a product of native phagocyte microbicidal action.

Chemiluminigenic Probing. Measurement of native CL provides a direct, nondestructive measure of phagocyte oxygenation activity. Use of high quantum yield chemiluminigenic probes, such as the cyclic hydrazide luminol (13) and the acridinium salt lucigenin (14), increases the sensitivity for detecting phagocyte oxygenation activity by >4 orders of magnitude with respect to the native system. Use of luminol and lucigenin also impose a differential specificity with regard to the type of dioxygenation activity measured. With regard to the PMNL, luminol-dependent CL is mainly, but not exclusively, a measure of myeloperoxidase activity. However, lucigenin-dependent CL requires reducing equivalents as well as O_2 and can be considered a reductive dioxygenation (15). Lucigenin CL can be inhibited by superoxide dismutase and provides a measure of superoxide generation by the phagocyte.

The sensitivity of the chemiluminigenic probe approach is so great that phagocyte activity can be measured with relatively good precision from submicroliter quantities of whole blood (3-4). Use of a very small number of phagocytes for testing serves to eliminate problems with regard to cell crowding, such as the rate-limiting effects of glucose and O_2 consumption and the accumulation of lactic acid.

The Humoral-Phagocyte Axis: An Information-Effector Mechanism. Acute immune protection from microbial infection is provided by the humoral system, composed of the complement pathways and microbe-specific immunoglobulins, and the PMNL. The humoral system identifies and labels the microbe and signals the PMNL. The PMNL responds to the chemotactic signals by migrating to the site of infection where contact with the immune-labeled microbe results in phagocytosis and activation of the respiratory burst metabolism required for microbicidal

dioxygenation, resulting in CL. Therefore, the kinetic relationship between the microbe, the humoral information system, and the phagocyte can be described by the empirical rate equation:

$$v = k\,[\text{Microbes}]^{n}[\text{Humoral Factors}]^{h}[\text{Phagocytes}]^{p}$$

where v is the CL intensity (i.e., velocity: dhv/dt, k is the proportionality constant), [ ] are the concentrations of the variables described, and the superscripts m, h, and p are the orders of the reaction with respect to the variables described (16,4).

With adequate control of the variable involved, it is possible to quantitatively measure the various components of the acute immune response as the resulting product CL. The sensitivity, in combination with the controllable broad-spectrum applicability of the chemiluminigenic probe approach, makes it ideal as an in vitro system for homologous analysis of in vivo inflammatory state and capacity. My present work is aimed at realizing and expanding these possibilities.

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THE USE OF INTRAVENOUS IMMUNOGLOBULIN G IN BURN PATIENTS

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Pseudomonas aeruginosa has a very limited capacity to produce infections in healthy people, but it readily invades compromised hosts with immunologic deficiencies, such as patients with thermal injury. Pseudomonas still remains a frequent and potentially lethal pathogen and immunotherapy represents a method of prevention and treatment of Pseudomonas infections in a variety of clinical conditions. The present study describes the results of an open clinical trial of intravenous tetravalent hyperimmune Pseudomonas-intravenous immunoglobulin G (THPG) in burn patients with Pseudomonas sepsis.

THPG was prepared from pooled human plasma collected from screened normal donors selected for high titer of naturally occuring antibodies to lipopolysaccharide antigens of Pseudomonas aeruginosa, Fisher-Devlin-Gnabasik immunotypes 1, 2, 4, and 6, identified by an enzyme-linked immunosorbent assay (ELISA) (1).

Patient eligibility criteria included any patient older than 6 mon and with any size burn if they had Pseudomonas aeruginosa bacteremia, clinical evidence of systemic Pseudomonas sepsis with the wound as the source, a quantitative wound biopsy of a 3° burn with $>10^5$ Pseudomonas organisms per gram of tissue, a 2° burn with heavy surface growth of Pseudomonas, or bronchopneumonia.

Ten patients were enrolled over a 9-mon period between January and September 1985. The mean age was 21 yr, with a range of 2-43 yr. The mean total body surface area burn size was 51%, with a range of 20-97%. The mean 3° portion was 30.8%, with a range of 0-97%. The mean postburn day of a positive burn wound culture for Pseudomonas was 7 days, with a range of 4-21 days. The mean postburn day of bacteremia was 14 days, with a range of 6-22 days. The sources of sepsis included the burn wound alone in 3 patients, pneumonia in 2 patients, and the burn wound and pneumonia in 5 patients. Eight of the 10 patients had documented bacteremia.

The dose of THPG was 500 mg/kg based on the patient's preburn weight. This amount was given for each of two successive infusions, which were separated by 24 h. The amount of THPG given ranged from 9.5-48 g/infusion. Immunoglobulin G (IgG) levels were obtained before, immediately after the conclusion of the first infusion, prior to the second infusion,

and then on postinfusion days 1, 3, 5, 7, and 14 after the last infusion.

The mean postburn day of the first THPG infusion was 17.7 days. Baseline IgG levels are shown in Table I and Figure 1. There was a statistically significant difference between the mean pre- and postinfusion IgG levels after the first and second infusions. Serotyping of the Pseudomonas isolates responsible for the bacteremias revealed that the common immunotypes were Fisher immunotypes 1, 2, 4, and 6. The mean pre- and postinfusion IgG ELISA antibody titers for the first and second infusions in the 10 patients for immunotypes 1, 2, 4, and 6 are shown in Table II.

TABLE I. Immunoglobulin G Levels vs. Infusion Time (mg/dl)

	Mean	Range
Baseline	1,014	577 - 1,690
Preinfusion 1	1,699	1,310 - 2,490
Preinfusion 2	1,536	823 - 2,240
Postinfusion 2	2,205	1,540 - 2,980

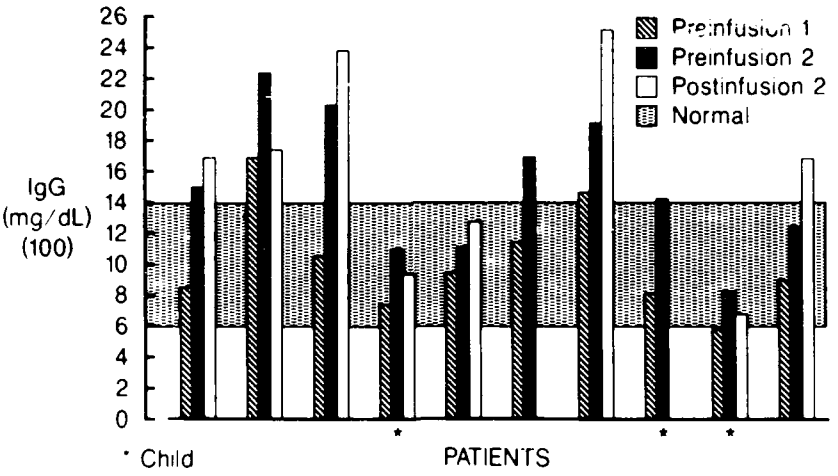

FIGURE 1. Immunoglobulin G (IgG) levels after infusion of Pseudomonas immune globuln.

Four patients were able to mount a significnt antibody response to the infecting Pseudomonas serotype. Their increase in the ELISA titers to immunotype 2 was to a level higher than could be attributed to the infusion of the THPG alone. Each patient survived. The improvement in their clinical course

TABLE II. Enzyme-Linked Immunosorbent Assay Titers to Lipopolysaccaride Immunotypes

	Immunotype							
	1		2		4		6	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Preinfusion 1	2845	126-7559	16739	3102-72526	4410	388-14156	4281	546- 9315
Postinfusion 2	8838	4501-19981	36449	13500--76841	24717	9128-35347	23698	4251-47128

corresponded to a 3- to 125-fold postinfusion increase in antibody titers. The increased titers were maintained as long as 3 wk postinfusion.

Recurrent Pseudomonas bacteremia was documented in 2 patients. One did not receive a repeat infusion and a subsequent blood culture was positive 7 days after the first infusion. In the other patient, the interval between positive cultures was 10 days after the first infusion. This patient was given a second infusion.

In 9 patients, the Pseudomonas was resistant to all currently available antibiotics. These patients were, nevertheless, given maximum recommended therapeutic doses of both an aminoglycoside and a semisynthetic penicillin. Three patients died. Their percent burns were 97%, 51%, and 80%. The initial source of sepsis was the wound in 2 followed within 6-8 days by the appearance of pneumonia. The other patient died of pneumonia. Complications arising either during or following the infusions of THPG were minor. Three patients developed glycosuria during and after each infusion.

The killing of the Pseudomonas organism requires not only the presence of functioning neutrophils, but also complement and Pseudomonas antibodies. Any alteration of the these results in insufficient protection (2). Abnormalities of neutrophil function are present after thermal injury and, in spite of normal circulating antibody (IgG), protection against Pseudomonas sepsis may be insufficient. Multiple complement abnormalities are also present in burn patients (3). When this occurs in the patient with abnormal neutrophil function, resistance to Pseudomonas sepsis is decreased.

The most important protection-inducing components of the Pseudomonas organism are its surface antigens, and IgG is the most important antibody against Pseudomonas. Immediately after thermal injury, IgG is depressed and reaches a nadir at about 48 h postburn. Generally, this depression is directly related to the percent surface area burned. It may take as long as 3 wk to several months to regain normal levels. Patients who have a severe depression of IgG at 48 h are at a high risk of dying (4). The great variability of the antibody titers to the 4 immunotypes after the THPG infusions between patients in this series may be due to a number of factors, including increased catabolism, decreased synthesis, fluid loss through the wound or during surgery, prior infection, and, of course, antibody consumption.

Because of the prevalence of Pseudomonas as a pathogen in burn patients, new avenues of therapy have been explored. Immunologic intervention appears to be very promising (5). Both active and passive immunization have been used intermittently since the late 1880s. The treatment of active,

ongoing infection in burn patients must involve passive immunization (6).

Numerous animal studies have shown that passive immunization against Pseudomonas sepsis is effective. Feller and Pierson (7) reported the results of both active and passive immunotherapy in burn patients. Passive immunization utilized plasma derived from human volunteers previously immunized with a monovalent vaccine. Comparison to retrospective control revealed the mortality attributed to both Pseudomonas sepsis and pneumonia decreased from 51% to 5% and 32% to 4%, respectively.

Jones et al gave 14 burn patients a hyperimmune globulin derived from plasmaphorescing patients that had been immunized with a polyvalent Pseudomonas vaccine (8). The globulin preparation was composed of antibodies against 7 immunotypes of Pseudomonas aeruginosa. They noted clinical improvement in most patients along with abrogation of the sepsis. An increase in the hemaglutination and antibody titers and improvement in opsonic activity of the serum was evident. Roe and Jones (9) used both active and passive immunization in burn patients with Pseudomonas infection in a hospital in India. Thirty-seven patients were given a 16-part vaccine and an immunoglobulin. Their mortality was 10.8% as compared with a mortality of 7.2% in 83 patients treated only with immunoglobulin prophylactically. One hundred and seventy-seven patients were treated by active immunization with a vaccine and their mortality was 8%. The mortality from Pseudomonas sepsis in a control group was 30.4%. Of interest was the emergence of Klebsiella aerogenes as the new burn pathogen.

Kuzin et al (10) reported using intravenous hyperimmune anti-Pseudomonas plasma in 10 patients. The plasma was obtained from voluntary donors injected with a cell-free vaccine composed of 5 serotypes isolated from their burn unit. It was highly effective in treating septic patients.

Loebl et al (11) treated 6 cases of ecthyma gangrenosum in pediatric burn patients with systemic (two times the normal recommended dose) and subeschar gentamicin and intravenous carbenicillin plus both Pseudomonas vaccine and hyperimmune globulin. The latter two were used in 4 patients and 1 survived. The globulin had no antibodies against the infecting strain of Pseudomonas. Three of the children survived.

In August 1986, all burn patients at Parkland Memorial Hospital (Dallas, TX) were moved into a new burn unit. Since that time, only one case of Pseudomonas bacteremia has occurred and wound colonization with Pseudomonas has decreased from 11% to 2%.

In this pilot study, THPG appeared to be a beneficial adjunct in patients with Pseudomonas bacteremia. ELISA

antibody titers rose and were sustained during clinical improvement and abrogation of sepsis. Although the number of patients studied was small, further clinical trials at other burn centers are warranted.

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POSTINJURY IMMUNOMODULATION

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THE PASSAGE of time has rendered the prospect of immune modulation more difficult rather than easier, because the immunological alterations that occur after thermal injury seem increasingly complex. For the purpose of this paper, "modulating agents" will mean biologic response modifiers acting on the cell-mediated immune system and on nonspecific responses.

The following approaches to modification of the immune response will be specifically excluded, not because they are not extremely important, but because they do not fit under the strict definition of "modulation": classic vaccination, both active and passive, the mechanical or biological removal of toxins, early wound closure, nutritional support, and replacement of depleted substances such as fibronectin.

The rationale or need for postinjury immunomodulation is dictated by convincing evidence of correlation between postburn immunosuppression and increased morbidity and mortality.

While the precise etiology of postburn immunosuppression is unknown, it would appear that the timetable of this immunosuppression occurs in two phases, each with its own underlying etiology: an early phase peaking at approximately 3 days postburn and a late phase peaking at 8-10 days postburn. These changes are illustrated in Figure 1.

If and when the patient becomes clinically septic, a third phase of suppression mediated by bacterial products is noted. The early phase is characterized by the appearance in the circulation and in the wound of mediators of the inflammatory reaction and of the "fight/flight" response. Included in this group are the opioid peptides, the prostaglandins, particularly of the E series, and corticosteroids. Toxic serum factors also appear during this phase, and endotoxemia of gastrointestinal origin due to increased mucosal permeability of the gut may also play an important role during this early phase. During the later phase, increased biological activity by suppressor T-cells has been documented, and failure of nutritional support as well as bacterial colonization of the wound may play a part.

A large number of biologically active agents are known to modulate the immune response, and over 400 of these biological response modifiers have been investigated *in vitro* and *in vivo* (1-4). They may be conveniently grouped as follows:

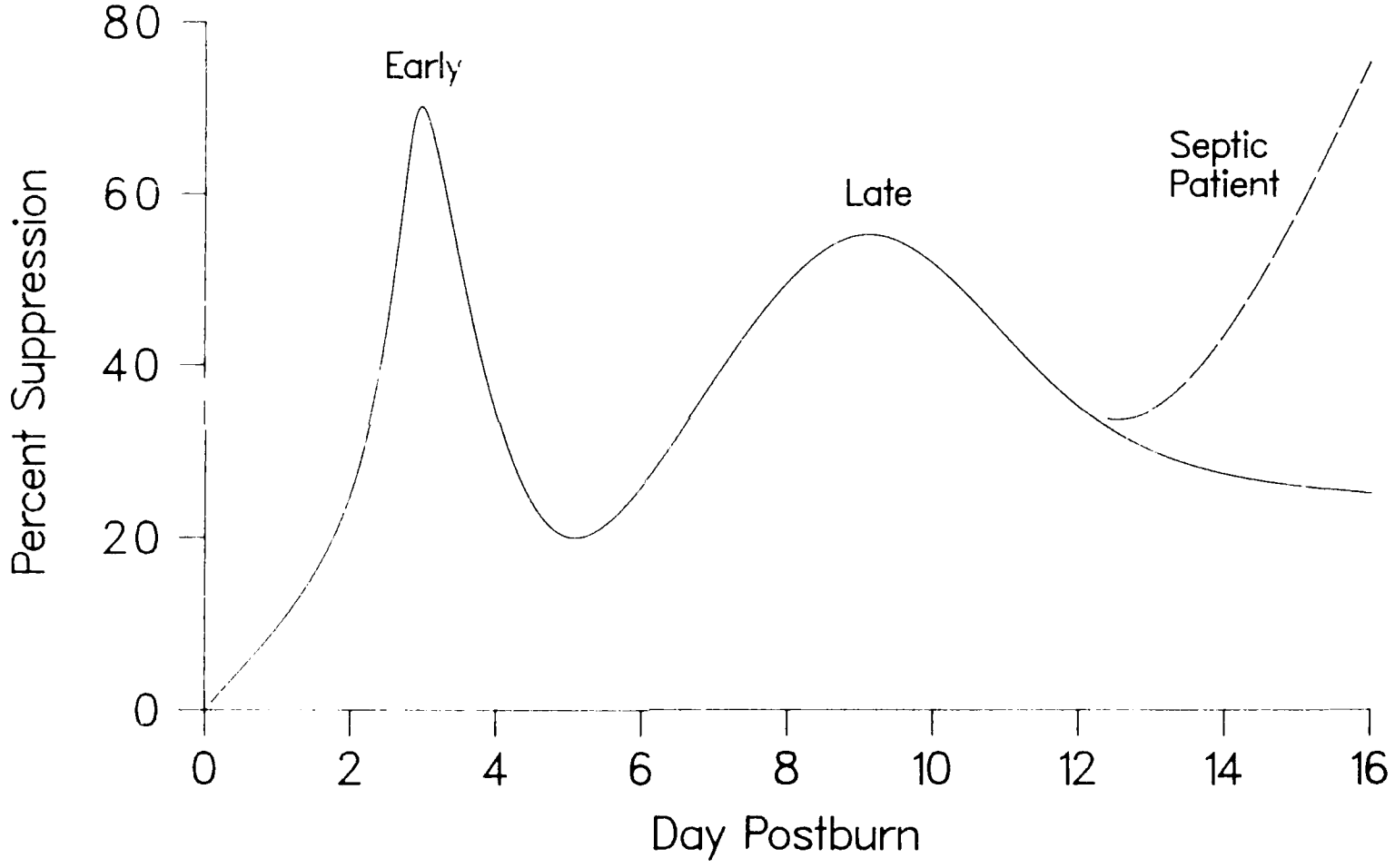

FIGURE 1. Phase of postburn immunosuppression.

1. Thymic factors, natural and synthetic.

2. Lymphokines interleukin 1, interleukin 2, and interferons.

3. Vitamins, particularly A, C, and E.

4. Agents that block the effect of endotoxins such as polymyxin B, $(Fab)'_2$ fragment, anticore antibody, and tolerin, which is a cobalt-irradiated form of endotoxin.

5. Biologic blocking agents, such as cyclophosphamide, ibuprofen, naltrexone hydrochloride, and cimetidine.

6. Intracellular polynucleotide messengers such as poly IC, poly EU, and poly ICLC.

7. Adjuvants, including Corynebacterium parvum, BCG, and MDP.

8. A large number of other substances which have been commercially developed or noted to have biological activity, including CP 4665, zymosan, pyran, glucan, methysoprinol, lithium, cerium, OK 432, BM 41-332, and MUE-2.

A wide variety of experimental settings ranging from improving the ear swelling of rodents following burns in response to DNCB (5) to mortality studies in dogs and guinea pigs, have indicated a positive role for many of these agents in modifying the immune response. However, a large number of difficulties remain between in vivo and in vitro laboratory findings and clinically convincing application in humans. These difficulties may be summarized as follows:

1. **Phase variations in response, such as seen with BCG, Corynebacterium parvum, endotoxin, vitamin E, and zymosan.** These agents are stimulatory in low doses and suppressant with high doses; some of these agents even have a triple-phase variation in response (6).

2. **The inability of immunomodulators to deal with toxic factors in the serum.**

3. **Dual action of certain agents, such as polymyxin B, which neutralize endotoxin (7).** Unpublished data from our laboratory indicate that treatment of patients with polymyxin B increases the interleukin 2 responsiveness of lymphocytes in vitro, but there is no change in interleukin 2 receptor shedding in the serum. This implies that endotoxin has several sites of action.

4. **Potential interference with the "fight/flight" response.** Presumably in a stress situation, the organism channels energies to organs deemed to be more vital than the

immune system and is prepared to take the consequences of immunosuppression after initial survival has occurred. Little is known about the effect of interfering with this early response.

5. **Marked and strong species variations in the action of immunomodulating materials.** Corynebacterium parvum is an example of an agent which elicits different postburn responses in rodents, guinea pigs, dogs, and presumably man.

6. **Unexpected effect of combinations of agents.** In a prospective randomized series in a burn center, polymyxin B produced dramatic improvement in in vitro immunological parameters and a noticeable improvement in sepsis rate and survival. The administration of intravenous immunoglobulin G produced minimal in vitro improvement in immunologic parameters, and slight, not statistically significant, improvement in the survival and sepsis rate; yet the combination of polymyxin B and immunoglobulin G produced worse results than either polymyxin B or immunoglobulin G alone, almost as though the immunoglobulin G had negated the effects of polymyxin B.

It is this author's opinion that, because of the wide diversity of sites of immunosuppression postinjury affecting different target cells by widely different mechanisms, it will not be possible to provide a single "magic bullet" to reverse posttraumatic immunosuppression. What will be necessary is to test, in strictly controlled randomized prospective trials, the most promising biological response modifiers in combination with each other, repletion techniques such as the administration of immunoglobulin G or fibronectin, and mechanical or other toxin removal techniques. In combination with these trials, sound principles of burn care, including early aggressive wound closure and nutritional maintenance, must be observed in order for immunomodulation to have an optimal effect.

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PLASMA PROTEIN RATIOS AS INDICATORS OF INFECTION IN SEVERELY INJURED PATIENTS

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MARKED ALTERATIONS in plasma protein concentrations have been repeatedly demonstrated to occur during a wide variety of injuries (1), systemic infections (2-3), and extensive inflammation (4). The increase in concentration of some plasma proteins appears to correlate positively with the extent of injury, the severity of the infection, or the degree of inflammation and, in some instances, plasma protein alterations have been used to monitor the course of treatment. The plasma protein changes with injury, infection, and inflammation would appear to be an intrinsic aspect of the host's response, in that increases in plasma proteins occur even in malnourished, protein-deficient, or trace metal-deficient patients and animals during inflammatory stress (5). The seemingly essential nature of the so-called "acute phase" protein response may arise from the fact that many plasma proteins appear to participate in wound healing and affect host resistance to infection. It is conceivable during severe injury that the pattern of the plasma protein response that is optimal to facilitate wound healing differs from that which would provide maximum resistance to infection. If this is so, there might be alterations in the concentrations of specific proteins or in the ratios of certain proteins which could provide additional information to physicians to help distinguish between severely injured and severely injured, infected patients.

MATERIALS AND METHODS

Extensive details concerning the burn patients and the categories to which they were assigned have previously been published (6) but are summarized here. Only patients for whom complete plasma protein data were available were included in this report and only peripheral vein values were used in the calculations. The three groups of patients had similar ages, weights, and mean total body surface area burn sizes (51%) and were studied at similar times postinjury. The noninfected burn patients were normotensive and hemodynamically stable, had no abnormalities in serum electrolytes, pH, osmolality, BUN, or creatinine, and no clinical or laboratory signs of systemic

infection prior to and during the period of study. The bacteremic burn patients were apparently also in stable condition, but did display clinical signs of infection with changes in mental status and/or ileus, and had positive bloodstream cultures before and during the period of study. The bacteremic burn patients with complications, in addition to positive bloodstream cultures and clinical signs of sepsis, also required mechanical ventilatory support and suffered some degree of renal impairment. Plasma protein determinations were made using a Hyland laser nephelometer and antisera provided by Hyland. The plasma protein values were not normally distributed as determined by a Kolomogorov-Smirnov one-sample test, so all comparisons were made by a signed rank Wilcoxon test or by ANOVA on the ranked values for each protein using the BMDP series of statistical programs (7). Values were considered to be significantly different if $P \leq 0.01$ The data were also subjected to stepwise linear discriminant function analysis to determine which combination of the minimal number of variables could be used to partition the patients into their respective groups.

RESULTS

The plasma proteins that were measured in serum samples taken from these well-characterized patients included the two major protease inhibitors in plasma, α_1-antitrypsin and α_2-macroglobulin; two major trace metal transport proteins, transferrin (iron) and α_2-macroglobulin (zinc); proteins considered to be microbialstatic, transferrin and haptoglobin; a protein shown to affect phagocytosis and platelet aggregation *in vitro* and found to affect drug and hormone distribution *in vivo*, α_1-acid glycoprotein; and a well-characterized indicator of injury, infection, and inflammation which has been shown *in vitro* to affect the immune response, C-reactive protein. IgM was also assayed as a measure of the immune response.

Table I shows that concentrations of α_1-acid glycoprotein (increased), haptoglobin (decreased), and IgM (decreased) changed significantly so as to allow differentiation between burned and burned-infected patients but, except for α_1-acid glycoprotein, did not allow one to distinguish between those patients burned and burned-infected with complications. Individually, none of these proteins allowed one to sort the burned-infected patients versus those without complications.

However, stepwise linear discriminant function analysis indicated that these patients can be partitioned into appropriate groups on the basis of α_1-acid glycoprotein and haptoglobin concentrations in combinations. Together they permit excellent differentiation of the burned group from the

TABLE I. Concentrations of Selected Plasma Proteins (Mean ± SEM)

	Patient Groups		
	Burned (n=6)	Burned-Infected (n=5)	Burned-Infected with Complications (n=5)
α_1-antitrypsin	629 ± 42 (447-727)	452 ± 79 (307-749)	692 ± 108 (517-1023)
α_1-acid glycoprotein	105 ± 20[a,b] (33-155)	282 ± 13 (234-306)	235 ± 37 (153-352)
C-reactive protein	26.8 ± 3.5[b] (17.8-39.8)	24.9 ± 3.7 (17.8-38.0)	14.1 ± 1.8 (7.5-18.0)
Haptoglobin	371 ± 41[a] (216-461)	144 ± 8 (115-160)	214 ± 44 (99-355)
Transferrin	176 ± 24 (98-238)	92 ± 5 (76-102)	117 ± 30 (59-234)
α_2-macroglobulin	136 ± 25 (72-212)	95 ± 24 (55-184)	76 ± 13 (46-113)
Immunoglobulin M	164 ± 40[c] (99-359)	33 ± 9 (12-63)	71 ± 28 (9-175)

Data were rank-ordered; ( ) indicates range. [a] $P \leq 0.001$ vs. burn-infected, ANOVA; [b] $P \leq 0.01$ vs. burned-infected with complications, ANOVA; [c] $P \leq 0.01$ vs. burned-infected, ANOVA.

two infected groups and reasonable differentiation between the two infected groups (Table II).

Noticing that the response of some proteins was to increase while others decreased in the presence of infection, the ratios of various pairs of proteins in each patient were calculated. Table III shows the only sets of ratios that were found to significantly differ in the presence of infection. The α_1-acid glycoprotein concentration had to be in the numerator for the ratio to be significant. This in part reflects the fact that only α_1-acid glycoprotein exhibited a significant difference between burn patients who were infected and those who were not, even in the presence of complications, but does not explain why this should be so. When IgM or α_2-macroglobulin concentration formed the denominator, one could distinguish patients with infection from those without, but could not differentiate between infected patients with and without complications. When either of the two microbialstatic proteins, haptoglobin or transferrin, are in the denominator, one cannot only use the resultant ratio to discriminate between noninfected and infected patients, but also sort infected patients with complications from those without complications.

Though it may be merely fortuitous that the two most effective plasma protein ratios for differentiating infected from noninfected injured patients employ the concentration of microbialstatic proteins in the denominator, these findings are consistent with the hypothesis that there are significant and diagnostically useful differences in the plasma protein response pattern to injury and to infection. Though these ratios appear to be useful in confirming the presence of infection in severely burned patients, additional studies will have to be done to determine if these ratios are of general usefulness in other instances of severe trauma and to what extent nutrient support, liver function, and/or tissue blood flow influence the concentration of these proteins. Moreover, longitudinal studies will have to be done to assess when thse ratios change relative to the onset of infection.

In point of fact, a longitudinal study has been done measuring the plasma protein responses of volunteers in a typhoid vaccine evaluation study (3). When one retrospectively analyzes the data and then plots the α_1-acid glycoprotein/transferrin ratio, it is clear that the 9 volunteers who become ill, as evidenced by an oral temperature over 100°F, had a significantly increased ratio from the first day of illness. Ten exposed volunteers who did not become ill showed no change in this ratio. Thus, the α_1-acid glycoprotein/transferrin ratio is a very sensitive indicator of the presence of infection in uninjured as well as severely injured patients.

TABLE II. Retrospective Grouping Based on Discriminant Analysis

	Burned	Burned-Infected	Burned-Infected with Complications	% Correct
Burned	6	-	-	100
Burned-Infected	-	4	1	80
Burned-Infected with Complications	-	1	4	80

The function consisting of α_1-acid glycoprotein and haptoglobin generated by discriminant analysis was used retrospectively to predict the category for each patient.

TABLE III. Plasma Protein Ratios (Mean ± SEM)

	Burned (n=6)	Burned-Infected (n=5)	Burned-Infected with Complications (n=5)
α_1-acid glycoprotein/ haptoglobin	0.273 ± 0.37[a,b] (0.153-0.392)	1.983 ± 0.176[c] (1.708-2.661)	1.165 ± 0.123 (0.923-1.545)
α_1-acid glycoprotein/ transferrin	0.572 ± 0.052[a,b] (0.337-0.697)	3.105 ± 0.232[c] (2.189-4.206)	2.188 ± 0.266 (1.504-2.772)
α_1-acid glycoprotein/ immunoglobulin M	0.766 ± 0.204[a,b] (0.231-1.582)	12.516 ± 3.945 (4.857-25.083)	6.477 ± 2.785 (2.011-17.000)
α_1-acid glycoprotein/ α_2-macroglobulin	0.775 ± 0.086[a,b] (0.440-1.046)	3.609 ± 0.718 (1.582-5.018)	3.262 ± 0.529 (1.835-5.100)

Data were rank-ordered; ( ) indicates range. [a] $P \leq 0.001$ vs. burn-infected, ANOVA; [b] $P \leq 0.001$ vs. burned-infected with complications, ANOVA; [c] $P \leq 0.01$ vs. burned-infected with complications, ANOVA.

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ETHICAL CONSIDERATIONS IN BURN CARE

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THE POLARIZATION and low ignition point characteristic of debates on this subject are usually attributed to the violence of the pathological and microsocial phenomena involved, i.e., the extremes of somatic and psychological injury, the threats to life, health, self-image, social, and financial well-being. But political and cultural events, and specifically several recent publications, have also contributed. A brief historical review of these more macrosocial influences may objectify and reduce the temperature of discussion; it might also throw light on future options.

Before the 1890s, American medicine was held in disdain and distrust by Europeans and Americans alike. Medicine was deduced from whatever overall theory of therapeutics held sway at the moment, e.g., herbal medicine, homeopathy, etc. Between the 1890s and 1960 with the advent of EKGs, vaccines, x-rays, antimicrobials, etc., medicine became scientific, effective, and, not incidentally, enormously powerful. It held ". . .unparallelled dominance in the world of rationality and power," but in the process reduced virtually all social and moral dimensions of care to biological categories. Informed consent did not exist as a concept or law until the 1950s and the "technological imperative" was virtually unchallenged. By the 1960s with Medicare and Medicaid, the medical profession's sovereignty began to diminish and the 1970s marked a time of "stunning loss of confidence in medicine." Medicine became seen as "overpaid, overspecialized, overbedded, overbuilt, and ineffective for the poor and rural populace" (1).

At this low point of trust in medicine, consumer rights advocates flourished, and the number of articles on bioethical subjects multiplied exponentially in the medical literature. In 1972, the Canterbury and Cobbs decisions as well as the American Hospital Association's "Patient's Bill of Rights" were published. In 1980-83, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research completed its work. These documents strongly supported the view that "the weighing of. . .risks against the. . .fears and hopes of the individual patient is not an expert skill" (2) and not the physician, but the patient or patient-surrogate has the "final authority to decide" about acceptance or rejection of therapy (3).

About this time between 1974 and 1978, the Don Cowart case became widely publicized via the educational tape "Please Let Me Die," stimulating many who saw the tape to speak strongly in

favor of patient autonomy. In 1977, Imbus and Zawacki recommended that "During the first few hours of hospitalization. . .while lucid and with sufficient information. . .," patients with burns so severe as to be considered without precedent for survival be asked to choose between a full therapeutic regimen and ordinary care. When patients were mentally incompetent on admission and expected to remain so, the socially designated next-of-kin was asked to speak for the patient (4).

In sharp contradiction to this approach, in 1978, the NIH consensus exercise on burn care stated that "Physical and/or emotional shock in the burned patient make it impossible for the victim to contribute to the early decision-making process" and therefore treatment should be initiated for all patients (5). In 1980, McCrady and Kahn shared this view, disapproving even any attempt to inform the patient since ". . .the physician's presentation of the alternatives. . .must influence, improperly, the patient's choice" (6). None of these authors, however, discussed the role of the surrogate or criteria of competence.

For better or worse, the literature after 1980 has reflected a retreat from the attitude of "treating all no matter what the patient or patient-surrogate may know or say." The second NIH Conference on supportive care in 1980 concluded that ". . .living wills may be construed as having taken such decisions out of the realm of professional judgment" and it might be ". . .reasonable (but not questionably cost effective) to conduct. . .studies to determine objectively the severely injured patient's mental capacities and ability to effect what could be termed informed consent" (7). In 1984 and 1986, Wachtel et al offered the option of "comfort care" to certain patients with high risk of death and poor quality of life, if competent, and to surrogates if they were not competent. However, details of the option apparently were not negotiated (8-9). In 1986, Hammond and Ward reported their experience in which the doctor and the patient-care team made (but the patient and/or family could veto) the decision not to resuscitate but "keep comfortable" patients with burns "overwhelming" (in the experience of a particular unit) (10). In none of these reports were the criteria of competence addressed.

Within these four most recent publications, there appear concessions that a living will may, and a competent patient/patient-surrogate veto generally does, have priority over professional recommendation. However, doubts about the competence and freedom of a nonprofessional choice still linger. Moreover, because some survivors occurred among those offered comfort care and because in one paper a >40% total body surface area burn in a >60 yr old individual was not distinguished from one which was overwhelming (10), fear

persists that progress in burn care might be stifled or even undone by approaches of this type.

A constructive response to the problem of deciding when consent is substantially competent and/or free should capitalize on what we have already learned about medicine's historical approaches to problem solving. A deductive approach has been historically counterproductive. Thus, deciding that patients a prior cannot be, and therefore never are, competent is antiempirical, closing rather than opening avenues of investigation. A reductive approach concludes that there are no moral, social, or psychological dimensions to the problem, only technical and biological, and therefore only physicians may properly make these decisions, since only they have the required technical expertise. This approach denies the need to consider the multiple facets of burn care the burn team was developed to meet. Moreover, it was just such neglect of the nontechnical dimensions of patient care in the past which played a major part in producing the "crisis of confidence" medicine is now enduring.

While practice, of course, may not parallel what is published, an inductive approach seems more compatible with the traditional values of medicine, only now extending the approach to the nonbiological as well as to the biological dimensions of care. It is encouraging to see that this inductive approach, this examination of experience and development of models for testing, is prominent in the latest publications noted above (8-10). It is also evident in other recent publications. Faden and Beauchamp (11), for example, offer models of autonomy, consent, and coercion that might be inductively tested. The most recent work of Imbus and Zawacki (12) offers for testing a step-by-step model seeking to maximize autonomy in decision making in the burn intensive care unit (Table I).

But might not testing of "comfort only" options be itself a dangerous step on a slippery slope leading to premature surrender to severe illness and the stifling of progress? The psychological force of this argument cannot be denied. It should be prominent among the reasons cited to a substantially competent patient or patient-surrogate when, even without precedent for survival, full therapy is recommended. We maintain, however, that the decision to accept or decline our recommendation, though a tragic decision, is the patient's or the patient-surrogate's, not ours. After all, "It's not medicine's responsibility to prevent tragedies by denying freedom, for that would be the greater tragedy" (13).

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TABLE I. Guide to Autonomous Decision Making by Patients When They are Still Competent**

PATIENTS	PROBLEM	APPROACH		
		*"These are the facts:"**	*"It is your right . . ."*	*"Please may we . . ."*
All	1. Permission to treat present illness	Diagnosis: Treatment: usual; recommended. We advise; patient decides	. . . to consent, to decline, to get a second opinion.	. . . have permission to treat your present illness as recommended?
	2. Release of information about present illness	Your diagnosis and treatment are privileged information and can be revealed only with your permission.	. . . to decide who is told what.	. . . have your instructions about telling what to whom?
	3. Decision making about changes in present illness	Changes may occur in your condition. A change in treatment may be appropriate.	. . . to be told of changes; to decline, consent, or get a second opinion about recommended changes in treatment.	. . . approach you about future changes as we are now about your present illness?
Critically Ill	4. Decision making should patient become incompetent	During some period in your illness, you may become so ill with fever, etc., that you cannot speak for yourself.	. . . to choose who should speak for you should you become unable to speak for yourself.	. . . have the name of your designee and ask if he will accept this role?
	5. Decision making should patient's condition change to one in which survival has been to our knowledge rare or unprecedented†	Your diagnosis is serious; the death rate in the past has been —.‡ We are committed to prolonging life and to rapid, efficient pro-life action regardless of the cost. This is true now and will remain so even should you take a turn for the worse and your condition become one in which, to our knowledge, survival has been rare or even without precedent. We will continue maximal treatment unless you or your chosen spokesperson tells us to stop.	. . . to receive maximal therapy no matter what, even should your condition change to one in which survival has been, to our knowledge, rare or even unprecedented. However, you may wish to decline maximal treatment at a certain point.	. . . have your instructions about continuing or stopping maximal treatment with respirators and machines, etc., should your condition change to one in which survival has been, to our knowledge, rare or unprecedented?

*To make proper decisions, competent patients must be told the facts. They may, however, be disenfranchised by their own denial should any but the gentlest and most sensitive exposition of facts be made. It will take time and repetition to make a point gently, but the truth must be available if the patient wants it. A patient's desire not to be told must also be respected. The patient-designated spokesperson should join the discussion at this point. Before continuing, questions such as, "Would you like to discuss this further?" and "Would you like to be more specific?" help gauge the patient's willingness to discuss these matters. **From Imbus SH and Zawacki BE (12). Table used with permission from the authors.

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SUMMARY OF CHAPTER V - IMMUNOLOGY AND HOST RESISTANCE

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THE YEAR 1968 witnessed the transition of command at the US Army Institute of Surgical Research (then the Surgical Research Unit) from Colonel John A. Moncrief, MD, to Colonel Basil A. Pruitt, Jr., MD. It was in the mid-60s under Drs. Moncrief, Mason, Lindberg, and Pruitt that much of the Institute's activities started to focus on infection and host resistance. Potent topical and systemic antimicrobials such as gentamicin were put into clinical use. It was also at that time that the immunosuppression of the burned patient was appreciated and the ability of adequate nutrition to correct, in part, that immunosuppression was recognized. The nine authors represented in this chapter did their work at the Institute between the early 60s and the present. Many have continued the work started at the Institute during the rest of their careers.

Dr. Alexander's presentation examined alteration of opsonic activity after burn injury. Opsonization is the coating of bacteria with complement and antibody which facilitates phagocytosis of the coated bacteria. C3, which is key in the opsonization process, can be activated through both the classical and alternative complement cascade pathways. Both complement and antibody are necessary for opsonization. Alexander examined systems deficient in complement and antibody (gamma globulin). He showed that passive serum infusion corrects complement activity, but only if opsonic activity is >10% of normal. Furthermore, infusion of gamma globulin fails to correct the defect in opsonization. This indicates that other factors play an important role in control of phagocytosis. These other controlling factors seem to be related to the activation of the alternative pathway of complement activation by the burn injury, resulting in production of C3b and C5a. C3b inhibits phagocytosis and chemotactic factor C5a causes neutropenia, induces thromboxane production, and reduces lymphocyte function.

Dr. Yurt's presentation examined some of the numerous inflammatory mediators which are released or synthesized at the site of the burn wound within moments of injury. Both chemical cascades, e.g., complement, coagulation, and angiotensin, and cell-derived products, e.g., interleukins and histamine, have been demonstrated to be active. The effect of these mediators may be due to their intrinsic activity, the quantity of mediator released, the mechanism of release, or the influence

the mediator has on other cells or organs. These inflammatory mediators have local effect on the burn wound and widespread effect on the rest of the body. Dr. Yurt then focused on the mediator histamine, quantifying its release in burns of different sizes and describing its systemic effect and how that may relate to infection susceptibility of individuals with large burns.

Dr. Warden's investigation of burn-related humoral immunosuppressants stemmed from the observation that serum from burned individuals suppresses the proliferation of mixed lymphocyte cultures. Numerous inflammatory mediators, as well as the elusive "burn toxin," all seem to contribute to this suppression.

The effect of burn injury and infection on lymphocyte subpopulations was studied in detail by Dr. Burleson. He found that the usual method of separating white cells by Ficoll-Hypaque gradients is inaccurate in burns because a large number of immature polymorphonuclear neutrophils contaminate the lymhocyte partition. Fluorescent-labeled monoclonal antibodies are used to mark the subpopulations. The cells are then analyzed by flow cytometry using light scatter gates to restrict nonlymphoid cells from the analysis. Using this method, Dr. Burleson showed that the postburn proportion of both helper T and suppressor T lymphocytes is decreased, whereas the B cell population remains constant. The helper/suppressor ratio remains normal in burned animals and decreases only in animals whose burns are infected.

The interleukins recently have been the focus of the mechanism of interactions among various types of white blood cells. Dr. Lempert, on the other hand, examined the effect of hydrogen peroxide (produced in micromolar amounts by polymorphonuclear neutrophils) on human peripheral-blood mononuclear cells and lymphocytes. Addition of micromolar amounts of hydrogen peroxide to concanavalin- or phytohemagglutinin-stimulated mixed lymphocyte cultures profoundly inhibits the lymphocyte response without affecting cell viability. He went on to examine other effects of small amounts of hydrogen peroxide on these cells. Finally, he demonstrates that addition of lipid antioxidants substantially blocks the inhibitory effect of hydrogen peroxide, suggesting that hydrogen peroxide inhibits T cell activation by inducing lipid peroxidation.

Dr. Allen examined the physical chemistry of oxygen in polymorphonuclear neutrophils. He postulates that oxygen exists in various energy states within the polymorphonuclear neutrophils. That is, the electron orbits of the oxygen molecules acquire various spin states which make them more and less reactive or "excited." The reaction of excited moieties with each other reduces the excited states and results in emission of photons which are evidenced by chemiluminescence.

Chemiluminescence of granulocytes increases dramatically in septic burned patients. Therefore, chemiluminescence is proposed as a laboratory method to assist in the diagnosis of sepsis.

A pilot study using a tetravalent, intravenous, hyperimmune Pseudomonas immunoglobulin in 10 burned patients with culture-proven Pseudomonas sepsis was presented by Dr. Hunt. In 9 of the 10 patients, the isolated Pseudomonas was resistant to all currently available antibiotics. Nevertheless, all patients were treated with maximal doses of antibiotics in addition to administration of the Pseudomonas immunoglobulin. The immunoglobulin infusions significantly increased immunoglobulin G levels in all patients. Dr. Hunt concluded that his study shows that administration of intravenous hyperimmune Pseudomonas immunoglobulin is a beneficial therapeutic adjunct in treating patients with Pseudomonas sepsis but acknowledged that his study is small and that further clinical trials are necessary.

Dr. Munster found that because the immunological alterations which occur after thermal injury are increasingly complex, the prospect of modulating the immune system has become more difficult. He described two peaks of postburn immunosuppression, one occurring at 3 days and the second at 8-10 days after thermal injury. If the patient became septic, a later third peak was delineated. The first peak was probably related to the wound mediators of the acute inflammatory reaction and to the hormones of the "fright/flight" response. A list of compounds which can influence the first peak was presented. The second peak was characterized by changes in the ratio and function of T helper and suppressor lymphocytes. Modulation of the second peak of immunosuppression was difficult because of the interaction of the various available immunomodulators. The third or septic peak may be launched by endotoxin. Dr. Munster concluded that it was unlikely that a single "magic bullet" will be found to reverse postburn immunosuppression. Rather, he believed that combinations of modifiers, restoration of depleted substances such as immunoglobulin G, and removal of suppressors and toxins must all be used in concert to restore adequate immuncompetence to the postinjury patient.

Dr. Powanda summarized his years of study of plasma levels of acute-phase reactive circulating proteins such as transferrin, haptoglobin, alpha-acid glycoprotein, and others. He questioned why injured mammals break down muscle protein to allow the liver to synthesize these acute-phase reactants. It seems that some of these plasma proteins may facilitate wound healing. He then questioned whether measurement of acute-phase reactants or ratios of those measurements may assist in differentiating individuals with burns from those with infected burns. It appears that the ratio of alpha-acid glycoprotein to haptoglobin and to transferrin may provide such information.

MECHANISMS OF POSTINJURY HYPERMETABOLISM

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SEVERE INJURY is characterized by a set of rather stereotypic responses. With restoration of circulatory volume, catabolic alterations occur. Net proteolysis is accelerated and this is manifested by the increased excretion of urinary nitrogen, which results in the rapid loss of body protein. Oxygen consumption rises and body fuel oxidation shifts toward the utilization of body fat. Blood glucose increases as hepatic gluconeogenesis is accelerated, providing essential fuel for reparative and inflammatory tissues to optimize host defenses and ensure wound repair.

These postinjury responses are associated in time with alterations in the secretion of a variety of hormones and the elaboration of various cytokines, products of the host's own cells. Studies in this laboratory over the past several years have involved the infusion or stimulation of these mediators in normal volunteers in order to assess the relative influence of individual factors on the posttraumatic response. To investigate the role of hormones as mediators of the metabolic response to injury, 9 normal male volunteers received a continuous 74-h infusion of the "stress" hormones cortisol, glucagon, and epinephrine (1). As a control, each subject received a saline infusion during another 4-day period. Diets were constant and matched on both occasions. Hormonal infusion achieved plasma concentrations similar to those observed following mild-moderate injury. With this alteration in the endocrine environment, significant hypermetabolism, negative nitrogen and potassium balances, glucose intolerance, hyperinsulinemia, insulin resistance, sodium retention, and peripheral leukocytosis were observed. Single hormone infusions indicated that these responses resulted from both additive and synergistic interaction of the hormones. Triple hormone infusion simulated many of the metabolic responses observed following mild-moderate injury and other catabolic illnesses.

To evaluate the role of inflammatory mediators, we administered the sterile inflammatory agent etiocholanolone daily for 3 days by intramuscular injection. The effects of this agent were studied alone (2) or during the simultaneous infusion of the hormonal mixture (3). Etiocholanolone injection alone resulted in inflammation, fever, leukocytosis, increased serum C-reactive protein concentration, hypoferremia, and increased plasma activity of interleukin 1. Plasma concentrations of the counterregulatory hormones were normal

and catabolic responses were not observed. When etiocholanolone was administered with hormonal infusion, there was a major interaction of the two stimuli, i.e., both mediators were necessary for the complete manifestation of the host response to injury.

To evaluate the effect of endotoxin on mediating these responses, 7 normal volunteers received Escherichia coli endotoxin (4 ng/kg) by intravenous infusion (4). Saline was given during the control arm of the study. Endotoxin administration produced a response similar to an acute febrile illness, with flu-like symptoms, fever, tachycardia, hypermetabolism, and stimulation of stress hormone release. These events occurred in time shortly after the cytokine tumor necrosis factor was detected in significant concentrations in the plasma (5). Significant plasma elevations in interleukin 1 and gamma interferon were not observed. Similar acute-phase and catabolic events could be reproduced in stable cancer patients receiving the intravenous infusion of the cytokine tumor necrosis factor (6).

Thus, it appears that cytokines can stimulate the hormonal response to injury and mediate many of the acute-phase responses that occur following tissue damage and inflammation. Endotoxin can initiate or facilitate the generation of these substances. The wound and/or the gastrointestinal tract may serve as the route of entry for bacteria or their products following injury. Strategies to reduce the entrance of endotoxin into the body and/or the use of blocking antibodies to endotoxin or to dampen the cytokine signals may be important approaches to modify the catabolic responses to injury.

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THE WOUND AND HYPERMETABOLISM

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AT THE 30th Anniversary Symposium of the US Army Institute of Surgical Research, Arthur D. Mason, Jr., MD, reviewed past research accomplishments and suggested areas which needed further investigation. In reviewing metabolic research, he pointed out that much was known about the efferent mediators of the hypermetabolic response to injury but very little about the afferent signals. For example, by 1977, Douglas W. Wilmore, MD, had demonstrated that catecholamines were the principal efferent mediators of postburn metabolism, but we were still embroiled in the controversy over thermal and nonthermal metabolic drives.

The three basic assumptions were that these afferent signals originate in the burn wound, that they travel via the circulation rather than by afferent nerves, and that they alter central nervous system control of metabolism. While no one knew what the afferents were, everyone believed they originated in the wound. The connection was obvious since the hypermetabolic response varied with wound size and decreased with wound coverage and healing. The best evidence supporting both the second and third assumptions was provided by Wilmore et al in the early 1970s (1). They found postburn hypermetabolism was unaffected by loss of wound innervation and concluded that afferent signals must travel via the circulation rather than on sensory nerves. Likewise, they reported that brain death or heavy morphine anesthesia eliminated postburn hypermetabolism and suggested that the central nervous system played a vital role in the meatbolic response to injury. The question is how much have we learned about the afferent mediators of postburn hypermetabolism in the last 10 yr. While the answer does not reside exclusively in my own research, it will serve as a springboard for discussion.

The search for afferent mediators would be difficult to complete in the burned patient. For this reason, several animal models were tested. The rat was chosen because it could tolerate moderate to large burns and responded in much the same way as the patient. It became both hypermetabolic and febrile and demonstrated an increased sensitivity to environmental cooling, but the hypermetabolism could not be eliminated by external heating (2). The effect of ambient temperature on metabolic rate was demonstrated in a single group of animals prior to injury with pelage intact, after removing 50% of the fur coat, and after a 50% total body surface area burn. Loss of the fur coat increased their metabolic response to environmental cooling, but raising ambient temperature brought the metabolic rate back to normal resting levels. In contrast,

the hypermetabolism of the burned animals could be reduced but not eliminated by environmental heating. It is the afferent mediators of this "temperature-independent" hypermetabolism which we seek.

Resting oxygen consumption ($\dot{V}O_2$) of the 30% total body surface area burned rat varied with the extent of infection (3). Nonbacteremic animals do not demonstrate a measureable increase in $\dot{V}O_2$ until the second week postburn, while bacteremic rats are markedly hypermetabolic throughout. There are also differences in core temperatures between the two groups. While the nonbacteremic rat may run a low grade fever, one sign of systemic infection is a sharp rise in central body temperature (in excess of 38.5°C, normal = 37°C).

Since the burn wound is never sterile during the hypermetabolic phase of injury and infection alone produces metabolic and neuroendocrine adjustments similar to those in thermal injury, we wondered whether bacteria and/or their products were important metabolic stimuli in the nonbacteremic rat.

To study the metabolic effects of localized burn wound infection, we seeded wounds with a mutant strain of Pseudomonas aeruginosa (Strain 1244). This relatively nonmotile bacteria rarely invades normal tissue and causes systemic infection. Our first study involved seeding 30% total body surface area wounds on 4 groups of rats immediately after injury (3). We treated the wounds of two groups with mafenide acetate and left the others untreated. Less than 10% of all animals became bacteremic and were removed from the study. The metabolic response to seeding was apparent by postburn days (PBD) 7-8 (earlier than had been the case in unseeded rats). Mafenide acetate treatment lowered $\dot{V}O_2$ of the seeded animals. This suggested that a reduction in wound bacterial content lowered the metabolic response of the treated animals. Wound cultures were not performed; therefore, we could not be certain that the anitmicrobial effects of mafenide acetate were confined to the wound.

To determine whether postburn hypermetabolism varied as a function of wound colonization, we compared the metabolic response of animals whose wounds were seeded with the nonvirulent Pseudomonas aeruginosa with rats whose wounds were not seeded but allowed to colonize spontaneously. Mafenide acetate was not used in order to avoid the issue of local versus systemic drug effects. Bacteriologic cultures were performed on 60% of each wound and bacterial content expressed in colony forming units per gram of wound (cfu/g). Seeding the burn resulted in a constant level of wound colonization (10^6 to 10^7 cfu/g) over the 2-wk period of observation (Table I). The

TABLE I. Effects of Burn Wound Colonization (Mean or Mean ± SEM)

Postburn Day	0	3-4	7-8	14-15
Unseeded Group				
Number of Animals	33	25	15	12
Log Wound Bacterial Count (cfu/g)	-	$<10^4$	10^5	10^6
Percentage Bacteremic	-	-	20	20
Oxygen Uptake (ml/h/g)	0.80 ± 0.01	0.88 ± 0.02	0.94 ± 0.02	1.15 ± 0.04
Colonic Temperature (°C)	36.9 ± 0.1	37.0 ± 0.1	37.0 ± 0.1	37.6 ± 0.1
Seeded Group				
Number of Animals	53	40	36	27
Log Wound Bacterial Count (cfu/g)	-	10^6	10^6	10^6
Percentage Bacteremic	-	20	20	60
Oxygen Uptake (ml/h/g)	0.80 ± 0.01	1.07 ± 0.02	1.11 ± 0.02	1.24 ± 0.02
Colonic Temperature (C)	36.9 ± 0.1	37.4 ± 0.1	37.4 ± 0.1	37.8 ± 0.1

unseeded wounds colonized more slowly, averaging $<10^4$ cfu/g on PBD days 3-4, 10^5 by PBD 7-8, and 10^6 on PBD 14-15. There was a significant difference in log wound bacterial count between the two groups during the first postburn week. None of the unseeded animals were bacteremic on PBD 3-4, while 20% of the seeded animals had positive blood and/or spleen cultures. At 1 wk, 20% of both groups were bacteremic and, at 2 wk, 60% of the seeded animals were bacteremic as compared to only 20% of the unseeded group. $\dot{V}O_2$ was elevated in both groups by PBD 3-4, but the seeded group was more hypermetabolic. $\dot{V}O_2$ remained higher in the seeded animals throughout the first week, but this difference disappeared by the end of the second week due to a marked rise in $\dot{V}O_2$ of the unseeded group. The seeded animals also ran a low-grade fever throughout, while the unseeded group only became febrile sometime after the first week.

Since systemic infection raises $\dot{V}O_2$ of this model, all bacteremic animals were excluded to emphasize the metabolic effects of wound microorganisms. The increase in $\dot{V}O_2$ of 44 nonbacteremic rats correlated with wound bacterial count ($r = 0.63$, $P < 0.001$). Variation in the data suggests that there are several factors affecting metabolic rate other than the number of **viable** bacteria in the wound. This explains why *Demling* et al (4) failed to identify any relationship between wound bacterial content and $\dot{V}O_2$ in only 15 nonburned sheep.

What have we learned about the afferent mediators of postburn hypermetabolism over the last 10 years? First, the data support the assumption that the wound is an important source of afferent signals. This is particularly interesting in the light of recent work in Cincinnati (5) where investigators demonstrated that early enteral feeding reduced the hypermetabolic response of burned guinea pigs. Since the early feeding also prevented the usual decline in intestinal mucosal mass, these investigators concluded that the extra metabolism of the unfed animals was a result of loss of the mucosal barrier and the subsequent escape of bacteria and endotoxin from the gastrointestinal tract. Others have shown that bacterial translocation across the gastrointestinal tract of rats increases with burn size (6). The wound seeding studies do not support or refute these results. They do suggest, however, that if gut endotoxin is an important afferent in nonbacteremic animals, afferent signals from the wound add to or potentiate those coming from the gut.

The second thing we have learned is that bacteria play a major role in determining the afferent signals coming from the wound. Resting $\dot{V}O_2$ of nonbacteremic, burned rats increased in a dose-response manner with increasing wound colonization,

whether bacterial growth developed gradually in a natural fashion or was created abruptly by seeding the wound at the time of injury.

Finally, the metabolic effects of bacteria appear as a continuum, beginning with a modest rise in resting $\dot{V}O_2$ when only a limited number of a bacteria are confined to the wound. As wound colonization progresses, there is a further increase in $\dot{V}O_2$ associated with a rise in body temperature. Wound invasion and systemic infection results in even greater hypermetabolism and fever. Metabolic and febrile responses to invasive burn wound infection are well known, but there is a tendency to consider nonbacteremic patients as "free of infection." The present data indicate that such a concept may be misleading, since localized bacterial contamination of the wound does have systemic metabolic consequences.

What is it that we still do not know regarding the afferents of postburn hypermetabolism? While bacteria are somehow involved, the exact nature of the afferent signal(s) has not been established. Since bacterial effects are evident in nonbacteremic animals, the circulating afferent is not the bacteria themselves, but their products (toxins) or products of host cells in response to bacteria. Chronic, low-dose endotoxin administration has been reported to increase the metabolic rate of uninjured rats (7). Since we are seeding burn wounds with Gram-negative bacteria, we wondered if endotoxin was the circulating afferent. Using the Limulus lysate assay, we were unable to detect endotoxin in the plasma (sensitive to 30 pg/ml) of the nonbacteremic, seeded animals (unpublished data). Seeding wounds with Staphylococcus epidermis produces the same degree of hypermetabolism as Gram-negative infections reported above (unpublished data). This suggests that endotoxin is not the only bacterial product capable of being an afferent mediator of triggering the production of other endogenous mediators.

The hypermetabolic effects of circulating endogenous mediators are equally unclear. Serum of burned patients contains endogenous pyrogen (8), presumably interleukin 1, and fever and many other acute-phase responses of the patient are explained by the activity of this small peptide. There are data, however, which suggest that interleukin 1 is not the hypermetabolic afferent. Wilmore et al (9), for example, have reported that an inflammatory agent known to trigger interleukin 1 production made normal subjects febrile and caused them to manifest many other acute-phase responses, but did not make them hypermetabolic. In addition, nonbacteremic burned animals are frequently hypermetabolic but not febrile. Therefore, the presence of interleukin 1 in burn serum and evidence of other systemic manifestations of this endogenous mediator do not establish its role in postburn hypermetabolism.

It goes without saying that since we do not know what the afferent signals are, we know little about how they cross the blood-brain barrier and affect central nervous system control of metabolism. It is doubtful that either endotoxin or interleukin 1 cross the blood-brain barrier, so a mechanism must be identified by which these or other circulating agents affect the central nervous system.

In summary, were Dr. Mason so inclined, he could once again say that we have much to learn about the afferent signals of postburn hypermetabolism.

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POSTBURN THYROID FUNCTION

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THE RECOVERY course of the severely burned patient is characterized by hypermetabolism. Sixty percent of metabolic regulation has been attributed to the thyroid hormone, triiodothyronine (T_3), in the noninjured state. We have studied thyroid function prospectively in 25 thermally injured patients. Significant suppression of serum concentrations of T_3 and elevation of serum concentrations of reverse T_3 were seen. In less severely injured patients, the free thyroxine (T_4) index and serum thyroid-stimulating hormone (TSH) concentrations remained within the normal range. In patients with burn sizes >50% of the total body surface area burn, serum concentrations of free T_4 and free T_3 are significantly depressed.

Alterations in peripheral thyroid hormone economy occur following a wide variety of disease states which are characterized by catabolism (1). A major unresolved question is whether this chemical hypothyroidism represents functional hypothyroidism requiring thyroid hormone replacement therapy or is a nonfunctional sequelae of severe illness. The free T_4 index and free T_3 index as well as dialyzable free T_4 and free T_3 are lower with greater burn size and are lower in nonsurviving than in surviving patients (2). A compensatory elevation of basal TSH is not seen, suggesting the possibility of pituitary or hypothalamic hypothyroidism (3). The TSH response to thyrotropin-releasing hormone is attenuated in nonsurviving compared with surviving burn patients with comparable injury. The serum reverse T_3 concentrations increase with increasing burn size (4).

The hypermetabolic response to burn injury appears independent of thyroid hormone concentrations. We have measured catecholamine concentrations in severely burned patients. Both plasma norepinephrine and epinephrine vary inversely with serum T_3. A reciprocal relationship between thyroid hormone and catecholamines has been previously described in thyroid disease states (5). Metabolic rate correlates positively with burn size and with elevated plasma norepinephrine and epinephrine concentration for several weeks following thermal injury. Lack of an augmented TSH concentration, low plasma T_3 concentrations, and presence of

hypermetabolism suggest that the reduced plasma free T_3 concentration does not indicate functional hypothyroidism, but may reflect the assumption of metabolic control by the sympathetic nervous system (6). When multiple regression analysis was performed on data from severely burned patients with log plasma norepinephrine as the dependent variable and T_3, percent total body surface area burned, and age in years as the independent variables, log NE = 2.901 + 0.011 (age in years) + 0.009 (percent total body surface area burn) - 0.016 (T3 in ng/dl), r^2 = 0.905 (7-8).

Because of our concern about the possibility that hypothyroidism was a factor in the survival of our patients, a randomized prospective treatment protocol with T_3 was undertaken in 28 men (age = 17-23 yr) burned in a single gasoline fire. The patients were entered into a prospective T_3 treatment protocol versus placebo administration. They were randomized with respect to burn size to provide two comparable groups. There was no difference in survival between the T_3-treated group and the group receiving placebo. It is of interest that the metabolic rate was not different between the two groups despite the administration of 200 mcg T_3 daily to the group receiving T_3. Because of these data, it is recommended that thyroid hormone replacement not be given to patients who have suffered severe burn injury and who exhibit alterations in thyroid function tests consistent with the euthyroid sick syndrome. We feel that thyroid hormone replacement should be reserved for those burn victims who were known to have hypothyroidism prior to their injury or who exhibit an elevated serum TSH concentration.

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CENTRAL NERVOUS SYSTEM ALTERATIONS IN FLOW-PHASE BURN INJURY

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ALTERATION of central nervous system (CNS) function during the postresuscitation flow phase of burn injury has received relatively little attention. The original basis (hypermetabolism) for interpreting the presence of CNS alterations has been expanded to include a number of hormonal systems.

The clinical characteristics of the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) were described in postresuscitated burn patients in 1956 by Soroff et al (1) who speculated that hyponatremia was associated with an osmoregulatory mechanism set at a lower than normal plasma tonicity. We had an opportunity to test that hypothesis with measurements of plasma antidiuretic hormone (2-3). Hyponatremic flow-phase burn patients without clinically detectable hypovolemia or other known causes of SIADH had circulating arginine vasopressin levels elevated inappropriately for their diluted plasma, but varying normally with alterations of plasma osmolality. The osmostat was apparently reset about 20 mosm/kg lower than normal. The hypothalamic location of the control center suggests a CNS alteration as a result of cutaneous burn injury. On the other hand, the reduced total peripheral resistance and elevated visceral blood flow typical of volume-expanded burn patients susceptible to hyponatremia suggest that the brain may be responding, at least in part, to reduced effective volume from the influence of mediators that dilate visceral and other beds.

The resetting of thermogenesis to a higher than normal level in burn patients (Fig 1) indicates a burn size-related alteration of hypothalamic function (4-5). The elevated excretion and blood levels of catecholamines in burn injury result from prolonged activation of the sympathetic nervous system, a function of the CNS. This sympthetic activity helps drive postburn hypermetabolism (4,6), apparently in conjunction with elevated circulating glucagon and cortisol but not growth hormone (7). Though most sympathetically dependent systems, e.g., cardiovascular function, lipolysis, oxygen consumption, and renin secretion, remain overactive, paradoxically, the nocturnal surge of serum melatonin is blunted (8). In burn patients excreting 2-3 L urine and >50 mEq Na+/day and having normal to elevated creatinine clearance, elevated plasma values of renin activity, angiotensins I and II, and aldosterone are volume sensitive, but apparently not volume dependent (9). Very elevated serum cortisol concentrations may exhibit normal rhythm timing and diminished amplitude, whereas lesser

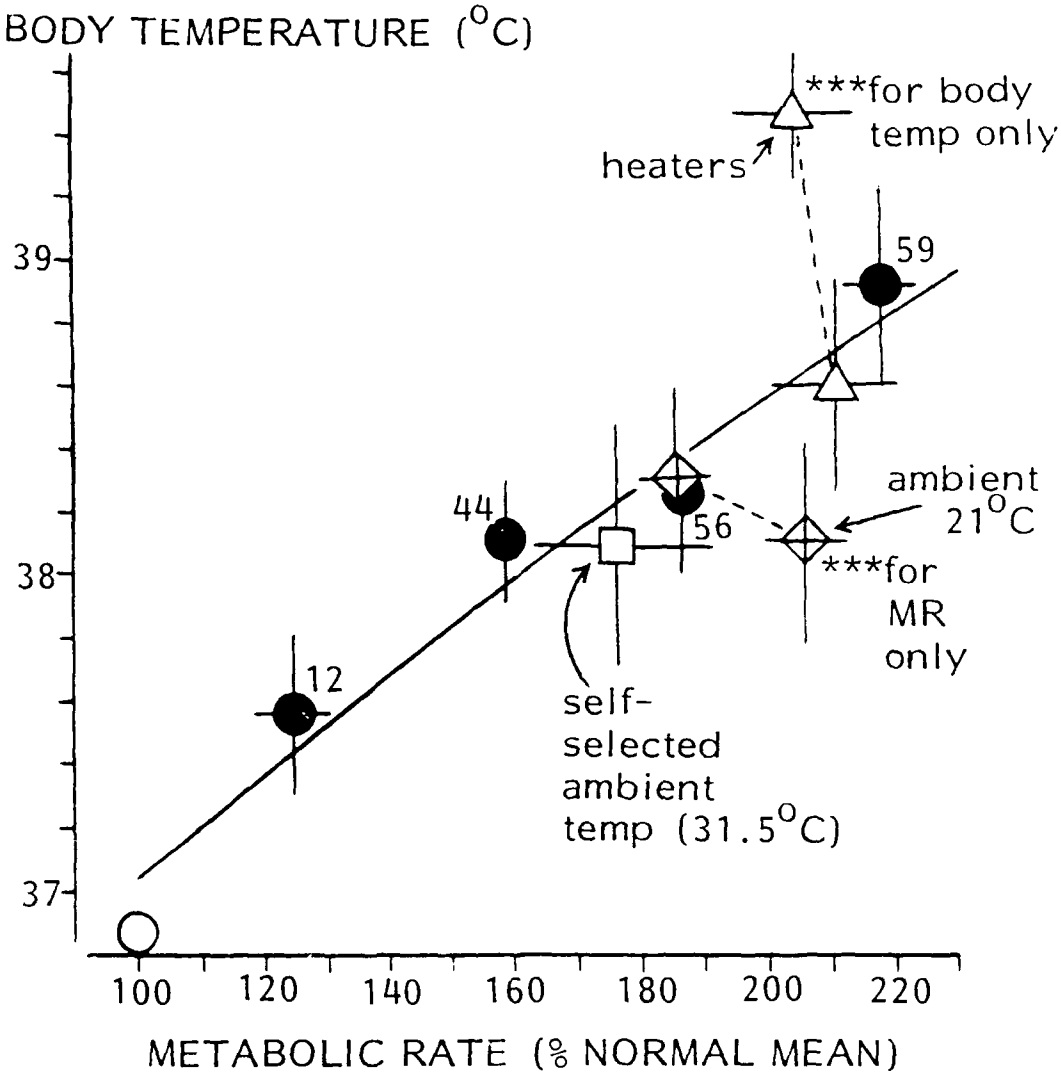

FIGURE 1. Thermogenesis at 30° or 33°C ambient temperature from data pooled between two reports (4-5) of adult fasting patients with mean total body surface area burn size given near the symbols (closed circles). Other groups were treated as indicated. The open circle near the origin is a group of healthy controls. Mean ± SE.

elevation may be associated with lack of a rhythmic fall to the normal low evening values (10). Rhythmicities of melatonin and cortisol are controlled by the CNS. The surges of growth hormone secretion that normally occur with nocturnal sleep (11) or after insulin-hypoglycemia or arginine infusion (12) (thought to be mediated by the hypothalamus) are blunted in burn patients.

Burned men have extremely depressed testosterone levels but often normal immunoactive luteinizing hormone (LH) levels. The very depressed serum bioactive LH (Fig 2) is evidence that the hypogonadism depends on a CNS alteration, a conclusion which is also reached by observation of the lack of major deficits of Leydig cell or luteotrophin responsiveness (10).

Depression of serum thyronines (thyroxine (T_4) and especially triiodothyronine (T_3)) may occur in burn patients, with greater decrements in nonsurvivors even for days to weeks prior to demise. Studies of thyrotrophin (TSH) with provocative testing (13) or with sensitive/specific basal measurements (14) indicate a deficiency of TSH which is more pronounced in nonsurvivors than in survivors. Although elevated dopamine and corticosteroid concentrations could contribute to TSH deficiency, the pattern suggests inadequate hypothalamic support of the thyrotrophin. Since immunoactive TSH concentrations are often normal, the deficiency can be interpreted as "relative", with TSH too low for the observed thyronine values. This lack of compensatory rise in TSH can be viewed as a resetting of the TSH-thyroid axis, such that given serum levels of thyronines have more than the usual amount of negative feedback effect (Fig 3), a condition that could result from a deficiency of TSH-releasing hormone from the CNS.

In study animals, this resetting is associated with measurable changes in the CNS. Its onset at postburn day 2 (Fig 4) coincides with the appearance of ectopic supraependymal neurons in the third ventricle just above the medial basal hypothalamus (15). Just as this resetting is characterized by relative failure of the pituitary TSH-secreting cells to recognize low serum thyronines as low, so also the brain fails to interpret the levels of serum thyronines as low in flow-phase burned rats (16). One of the best indices of the normal rat brain response to a deficit of serum thyroid hormones is the rise in type II 5'-monodeiodinase activity measured in vitro as a rise in the brain's abilitiy to convert T_4 to T_3 locally. Despite burn-induced depression of serum T_4 at least as pronounced as that produced by thyroidectomy (and depression of T_3), the elevated whole brain 5'-monodeiodinase seen after thyroidectomy was not seen after burn injury.

The CNS occupies a central position in the flow-phase hormone-metabolic response to large cutaneous burn injury. Whereas the afferent limb may involve cytokines from the wound influencing CNS function, the efferent limb appears to involve hormonal systems that participate in the hypermetabolism as well as other hormonal systems. A functional common denominator in these changes appears to be a resetting of control mechanisms that continue to operate.

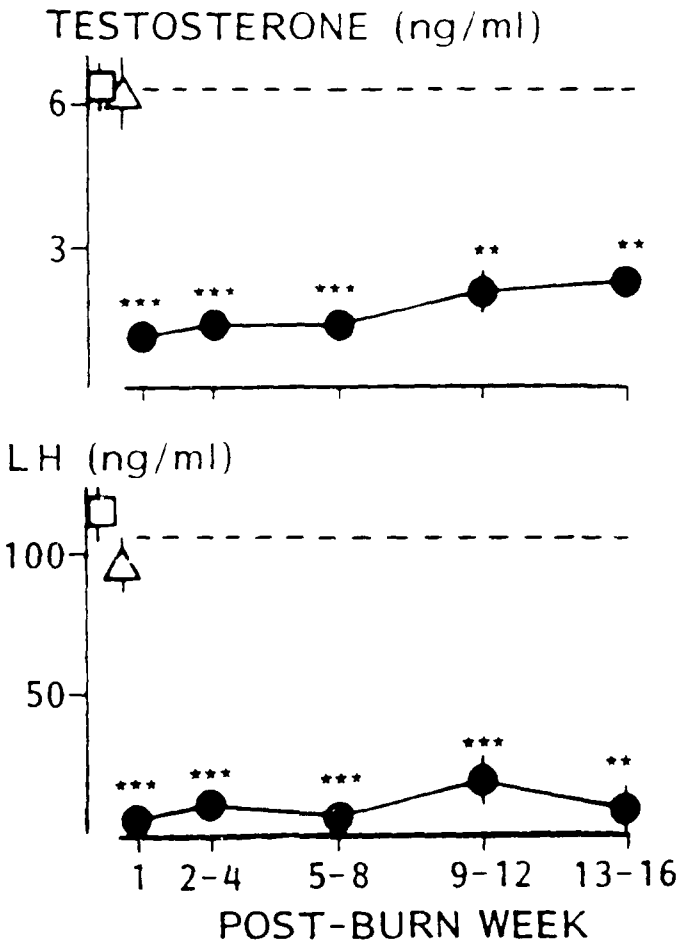

FIGURE 2. Serum testosterone and luteinizing hormone (by bioassay) in adult burned men (closed circles). Mean burn size (% total body surface area) for the various postburn time groups ranged from 37% (early) to 71% (late) and respective mean ages ranged from 39-24 yr. Open symbols represent healthy controls with mean age 25 (squares) and 59 (triangles) yr. Mean ± SE. **P<0.01, ***P<0.001 vs. either control group (17).

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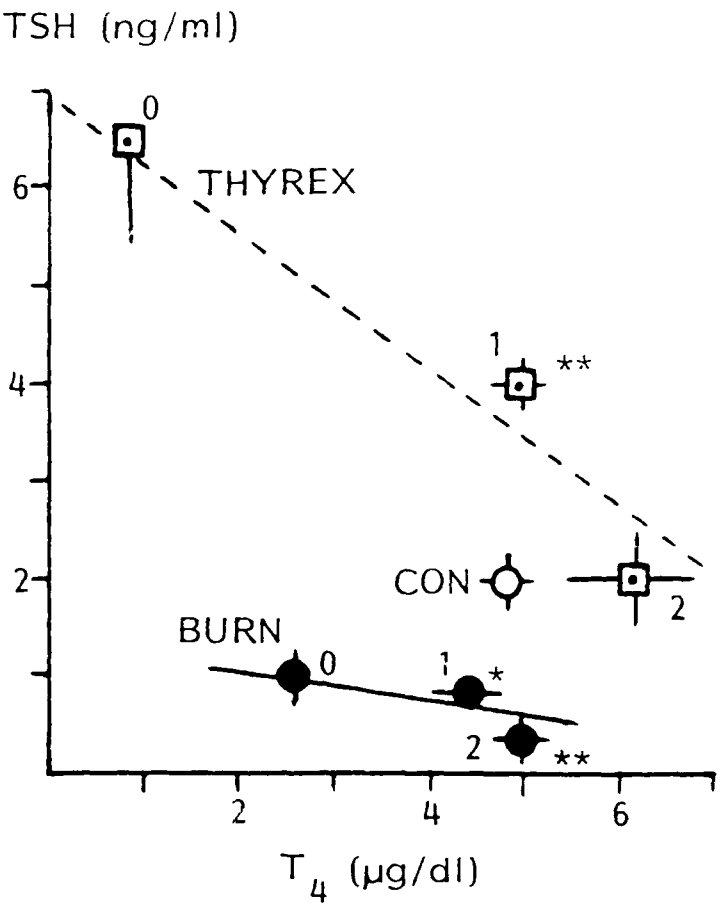

FIGURE 3. Serum thyrotrophin (TSH) and thyroxine (T_4) in rats at the end of 6 days with one of two concentrations of T_4 infused subcutaneously or no T_4 (indicated by the numbers near the symbols). Rats were sampled on postburn day 8 (full-thickness 50% BURN) or 12 days after thyroidectomy (THYREX). Mean ± SE. *P<0.05, **P<0.01 vs. unmanipulated controls (CON). TSH was measured with the NIH antibody by MK Vaughan. The known 35% elevation of the T_4 dialyzable fraction in burn rats (18) would not have elevated free T_4 to that of CON.

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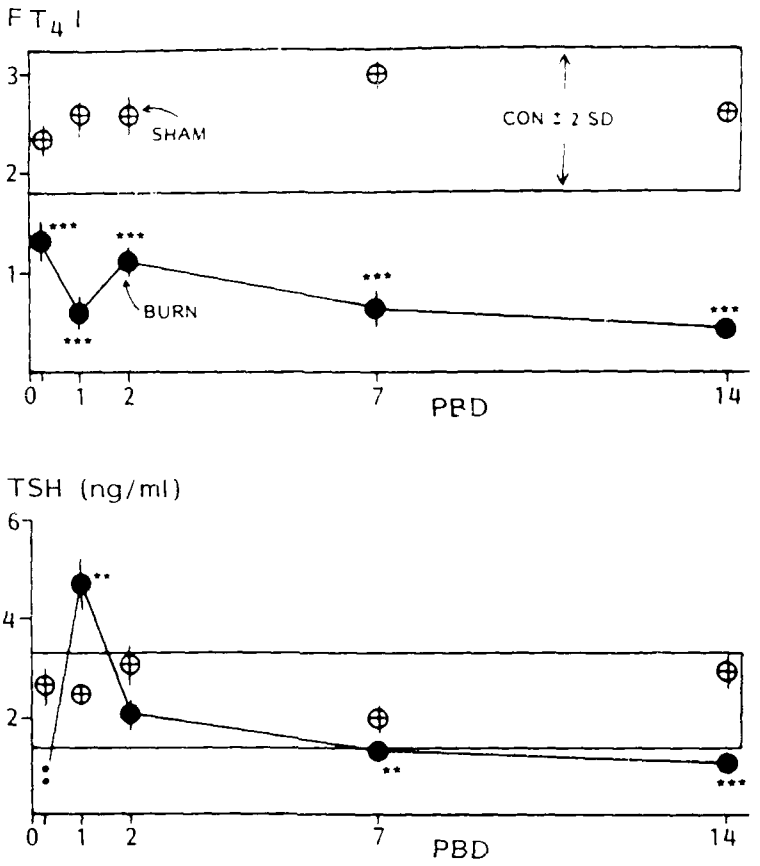

FIGURE 4. Serum free thyroxine index (FT_4I, thyroxine X *in vitro* triiodothyronine uptake) and thyrotrophin (TSH) (NIH antibody, MK Vaughan) in sham burned rats (SHAM) and rats with 60% full-thickness total body surface scald burn (BURN) by postburn day (PBD). Boxes represent vertical normal ranges from controls (CON). BURN and SHAM rats received 0.15M NaCl IP just before exposure of the ventral side. Mean ± SE. **P<0.01, ***P<0.001 BURN vs. SHAM. The fall of TSH on PBD 2 indicates resetting of the axis. From that time on, ectopic neurons appeared on the ventricular surface of the medial basal hypothalamus only in BURN (15). Although the FT_4I is the best indicator of thyroxine available to cells, total and dialyzable free thyroxine and triiodothyronine concentrations are also depressed on PBD 8 and 14 in 60% burned rats (18).

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LIPID UTILIZATION AFTER INJURY

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LIPID UTILIZATION as endogenous fuel to maintain energy homeostasis after injury was proposed many years ago and described in detail in Dr. Francis Moore's classic textbook on surgical metabolism almost three decades ago (1). More recently, isotopically labeled fat emulsion has been used to demonstrate that at least some infused fat is oxidized to carbon dioxide and water, presumably by beta oxidation (2). The rate of such oxidation, however, suggests that fat utilization is not dramatically accelerated after injury but continues at a rate only slightly greater than during resting starvation. A related but different question concerning fat utilization is how fat affects protein metabolism during simple starvation, after trauma, or during sepsis. This latter issue was the subject of investigation during 1972-75 at the US Army Institute of Surgical Research (San Antonio, TX) and subsequently at the University of Texas Medical School (Houston, TX).

The effects of carbohydrate and fat on nitrogen excretion were evaluated in five moderately to severely stressed patients, using a matrix study design in which each patient received at least three different combinations of fat and carbohydrate during isonitrogenous, intravenous feedings (3). Nitrogen excretion decreased significantly as carbohydrate (glucose) intake increased, but fat (soybean oil emulsion) did not affect nitrogen at any level of carbohydrate intake. Nitrogen excretion reached a plateau at approximately the point where carbohydrate intake equalled resting metabolic rate (Fig 1). Above that point, more carbohydrate did not cause further nitrogen-sparing, unless exogenous insulin was given to treat hyperglycemia, suggesting that the ability of carbohydrate to spare protein may be related to insulin availability.

Also in this study, nitrogen excretion was directly related to resting metabolic rate. The lack of protein sparing by fat was somewhat unexpected but not illogical, since oxidation of fat does not have a feedback mechanism to modify the demand for glucose during stress and ketosis is prevented by the stress response as well as glucose infusion.

Another study at the US Army Institue of Surgical Research used oral diets to compare carbohydrate and fat in hypermetabolic burn patients and normal controls during 7- to 10-day study periods (4). Again, nitrogen excretion was inversely related to carbohydrate intake and directly related

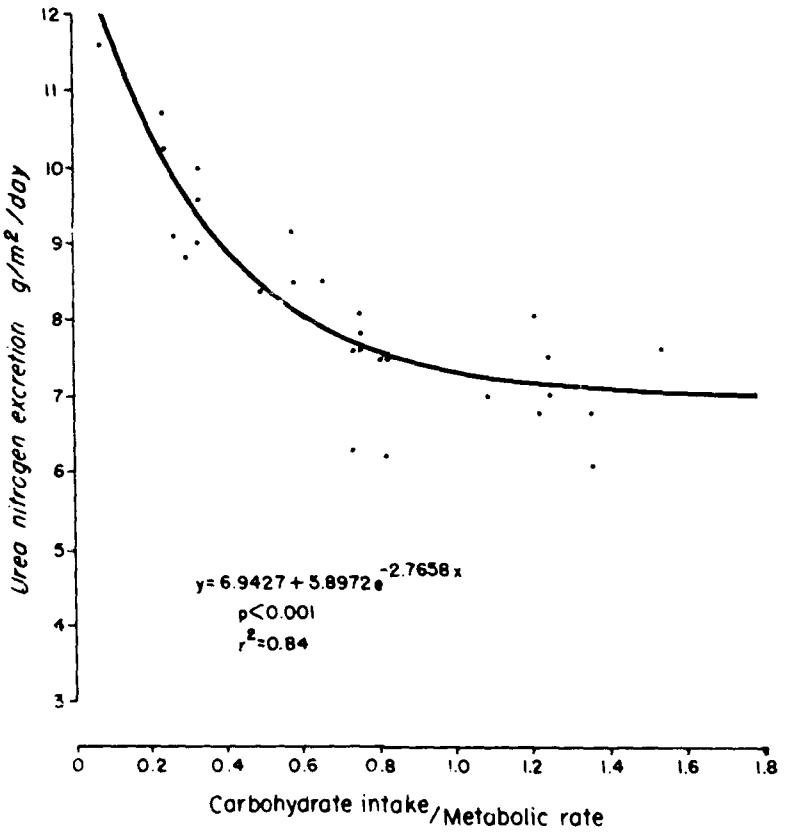

FIGURE 1. Nitrogen excretion decreased as carbohydrate intake increased, reaching a plateau near energy equilibrium. Fat did not affect nitrogen excretion.

to metabolic rate. Neither normal controls nor hypermetabolic subjects showed a nitrogen-sparing effect of fat.

Several investigators responded to the conclusion that fat did not spare protein, with contrary reports suggesting that 40-83% of nonprotein calories could be given as fat without adverse effects on nitrogen balance (5-7). Macfie et al measured total body nitrogen using neutron activation techniques and asserted that nitrogen accretion was greater when mixed substrates (50% carbohydrate and 50% fat) were infused than when all nonprotein calories were given as glucose (8). On the contrary, Shizgal et al using measurements of total exchangeable sodium and potassium, showed greater accumulation of body cell mass when glucose was the dominant exogenous fuel source (9).

Interpretation of data and reconciliation of apparently conflicting studies on the protein-sparing effect of carbohydrate and fat are quite difficult because of differences

of study design, variations of nitrogen and total calorie intake, and other variations of the feeding program. Other factors, such as failure to measure or report metabolic rate, could render many of the studies invalid, since variations of metabolic rate are well known to cause significant variations of nitrogen excretion.

Subsequently at the University of Texas Medical School, the intravenous rat model developed by Steiger et al (10) was used to further elucidate the relative protein-sparing effects of carbohydrate and fat. In healthy, growing Sprague-Dawley rats, nitrogen excretion was inversely related to both carbohydrate and fat intake (11). Although the mechanism by which fat caused apparent nitrogen-sparing could not be identified, the effect was roughly comparable to carbohydrate. These observations are similar to clinical experience with human infants, in whom the demand for calories and nutrients to support growth and development exceeds that of mature animals or adult human beings and in whom fat may appear to produce a protein-sparing effect.

When the rats were subjected to 40% total body surface area scald burns, nitrogen excretion increased significantly, confirming the magnitude of stress. Increasing carbohydrate intake caused a decrease of nitrogen excretion, but with this level of burn stress, the apparent nitrogen-sparing effect of fat disappeared. Stress blunted slightly the nitrogen-sparing effect of carbohydrate, but eliminated any appearance of protein sparing by fat.

Other investigators have confirmed limitations of the protein-sparing effect of fat emulsion during stress. Alexander et al (12) showed a detrimental effect on protein-sparing if fat represented more than 30% of total calories in stressed guinea pigs. Wolfe and Goodenough (13) showed that exogenous fat essentially replenished body fat stores, giving further credence to our earlier assertion that in stressed subjects, "fat spares fat." Baxter et al (14) have expressed caution about using fat emulsions after injury because of potential toxicity of long-chain fatty acids which are already elevated in the plasma after injury. A relative deficiency of carnitine has been proposed as a mechanism for this sustained elevation of free fatty acids and the failure of fat oxidation to increase appreciably during severe stress.

Concern has also been expressed about blocking of the reticuloendothelial system by fat emulsion (15), but adverse clinical effects have not been observed with dosages of fat below 90 g/day. Practically, most patients can be fed appropriately within their limitation of glucose oxidation if 50 g of fat are included in the diet, representing 20% or less of the nonprotein calories needed to meet energy equilibrium in injured subjects.

In conclusion, research and clinical experience indicates that exogenously administered fat emulsion, like endogenous adipose tissue, is utilized primarily by direct oxidation of fatty acids, but fat does not have a protein-sparing effect during stress. Fat infusion does serve to replenish body lipid stores. When limited to 50 g of fat emulsion per day in the average-sized adult, the potential adverse effects of fat emulsions or free fatty acids are avoided.

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CELLULAR METABOLISM AFTER BURN INJURY

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FOLLOWING major thermal injury and successsful resuscitation, total body blood flow increases and the injured subject demonstrates a marked increase in metabolic processes. The hypermetabolic response presents with increased total body oxygen consumption, negative nitrogen balance, and marked weight loss in the patient not receiving intensive nutritional support. This hypermetabolic response is proportional to the size of injury and is mediated in part by adrenergic neurohumoral mechanisms (1). A major determinant of this response appears to be the metabolic needs of the burn wound, which primarily utilizes anaerobic glycolysis to provide energy substrates for wound repair (2). Wound glucose is broken down to lactate and pyruvate, and these three carbon fragments are transported to the liver where new glucose is resynthesized from these components (Cori cycle).

The liver is one of the major organs involved in the metabolic responses to injury. Gluconeogenesis from lactate, pyruvate, amino acids, and other carbon skeletons occurs primarily in this organ, and liver dysfunction following injury is associated with increased morbidity and mortality. Liver blood flow and substrate flux have been measured by flow dilution techniques and arterial-venous differences from catheters inserted in a major artery and hepatic veins. Following burn injury, hepatic blood flow nearly doubles, as does oxygen consumption (3). Hepatic lactate and pyruvate uptake rise almost four times and glucose production nearly doubles. Because the liver is such a metabolically active organ following massive burns, the cellular mechanisms responsible for the increased oxygen and substrate utilization were investigated.

An established rat model of postburn hypermetabolism was utilized for this study (4). Following a 60% full-thickness thermal injury, the metabolic rate progressively increases until it reaches a peak 40% above baseline levels 10-12 days following injury. Liver tissue was obtained on postburn day 10, immediately placed in ice cold buffer, minced, and homogenized. Mitochondria were isolated by differential centrifugation and oxygen consumption was measured by polarographic electrodes. Control animals fed reduced amounts of food to produce the same weight loss experienced by injured animals were also studied. Isolated liver mitochondria exhibited increased oxygen utilization capacity when compared to control and partially starved animals (Table I). The respiratory control ratios (RCR) in all groups were similar.

TABLE I. Mitochondrial Function: Glutamate and Malate As Substrate (30°C)

	Control Group (n=12)	Partially Starved Group (n=12)	Burned Group (n=12)
State 3 ($nmoles/O_2/min/mg$ protein)	38.6	39.8	50.4*
Respiratory control rate (state 3/state 4)	6.6	6.9	7.0
Phosphorylation rate (nmoles ATP/min/mg protein)	220.0	221.8	287.2*
Weight change (%)	+4	-11	-12

*$P < 0.01$, control vs. burn group.

The rate of ATP formation was increased in injured animals. Thus, liver mitochondria demonstrate an apparent injury-induced capacity to carry out oxygen-requiring biochemical reactions. One of these functions is to manufacture elevated quantities of ATP, presumably for energy-consuming reparative processes. However, as evidence of normal RCR, the mitochondria remain intact biochemically and are not "uncoupled." Such a mechanism, if present, could explain the increased oxygen consumption and temperature production associated with the hypermetabolic response. However, such uncoupling does not occur, and mitochondria in this organ appear to function at an accelerated level with intact mechanisms. Thus, there appears to be no increased wastage of energy by excessive heat production.

Table I demonstrates mitochondrial function using energy substrates that enter the electron transport chain at Site 1 (NADH-linked reactions). Additional studies were carried out using fatty acids of varying chain length as energy substrates. These studies are of potential clinical relevance since several lipid nutritional solutions contain long-chain saturated and unsaturated fatty acids. Hypermetabolic-injured animals demonstrated a reduced ability to utilize long-chain unsaturated fatty acids (C18:2) and an increased ability to use a medium-chain fatty acid (C8:0) (Table II). Thus, a reformulation of currently available lipid formulas may provide better nutritional support for severely injured patients.

TABLE II. Effect of Fatty Acid Chain Composition on Mitochondrial Beta-Oxidation

	Control Group (n=12)	Partially Starved Group (n=12)	Burned Group (n=12)
C8:0	83.3	81.0	99.0*
C16:0	143.6	135.5	144.6
C18:2	144.2	152.2	121.8*

*P < 0.5. Fatty acid substrates assayed as acylcarnitine and rates expressed in nmole ferricyanide/min/mg protein.

Finally, preliminary studies have looked at the role of the terminal oxidase in the mitochondrial electron transport chain. Cyotchrome aa_3 concentrations were measured by dual wavelength spectrophotometry (5). Cytochrome aa_3 concentrations were elevated following injury (0.156 nmoles/mg protein in control animals, 0.154 in partially starved animals, and 0.199 in

burned animals). This elevation was proportional to the increased oxygen utilization. Turnover numbers of oxygen by cytochrome aa_3 were similar all three study groups.

These results indicate that mitochondria develop a hypermetabolic response to injury which parallels that of the intact animal. The cellular processes appear to operate, in part, by an increase in the capacity of the normal biochemical process. In the case of the increased state 3 oxygen utilization rates, additional cytochrome aa_3 appears to be synthesized by the mitochondria to meet these accelerated energy-producing reactions.

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